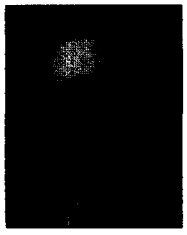
The Changing Scene of Surgical Gastroenterology—Some Reflections Gleaned From the Past 60 Years



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In the mid-1940s a major milestone was reached in terms of progress in the field of surgery. Far-reaching discoveries, which included a sound concept of surgical shock, development of techniques that would make blood transfusions readily available, the introduction of powerful antibiotics, and dramatic advances in anesthetic management along with vastly improved anesthetic agents, all led to an increased margin of safety in major operations. Significant technologic advances soon followed in the wake of these fundamental surgical adjuncts. This review will trace the evolution of surgical gastroenterology over the past 60 years, focusing on specific areas of the gastrointestinal tract along with associated diseases, complications, and other abnormalities.

STOMACH

Sixty years ago gastric surgery was one of the most prominent components of general surgery. Professor Hans Finsterer, a well-known Viennese surgeon, had just visited the United States and demonstrated his technique of partial gastrectomy to treat duodenal ulcers. Gastroenterostomy was still widely used despite the growing recognition that anastomotic ulceration was at times associated with the procedure. Distal gastric resection was becoming the standard operation at most major medical centers where it was rare for even one week to pass without several such procedures being performed.

A stressful lifestyle was considered the most common etiologic factor, and the mainstay of medical treatment was a bland diet that included large quantities of milk and tincture of belladonna four times a day. H_2 blockers and the recognition of *Helicobacter pylori* were, of course, years away. As the incidence of recurrent ulcers following resection began to mount, the extent of these resections was continually increased. Seven-eighths and nine-tenths gastrectomy procedures were even proposed, but fortunately these were never widely accepted.

The effectiveness of Dragstedt's vagotomy as compared to partial resection was a hotly debated subject during the late 1940s and early 1950s; however, as the procedure became widely accepted, attention turned toward attempts to improve results and lessen side effects by restricting the vagotomy to an increasingly limited section of the nerve ending with the use of the so-called "parietal cell vagotomy."

At approximately the same time that operative techniques of acid reduction reached a high level of acceptance, vastly improved antacid medications also became available; thus the incidence of operative treatment of peptic ulcers has plummeted since the 1950s and 1960s. Treatment of *Helicobacter pylori* infection also seems to have lessened the need for surgical treatment.

The standard indications for surgical treatment, which formerly included bleeding, perforation, obstruction, and, most common of all, intractable pain, have now been more or less limited to perforation, obstruction, and refractory bleeding. Pain refractory to all forms of medical treatment today is rare.

The uncommon form of peptic ulceration associated with the Zollinger-Ellison gastrinoma can now usually be controlled by removal of the tumor or pharmacologic reduction of acid secretion without resorting to total gastrectomy as recommended in early reports.

The incidence of stomach cancer in the United States has declined so consistently and dramatically

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since the 1930s, when it was the most common cause of cancer death, that gastric resection for cancer is no longer the common procedure it was. For example, it was reported that 62 gastric resections for stomach cancer were performed at the University of Minnesota in one year (1945). The cause or causes of this decline are still a bit of a mystery, although there is much evidence to suggest that it is related to dietary factors and possibly to a lower incidence of *Helicobacter pylori* infection.

After a brief trial using total gastrectomy to treat stomach cancer, high subtotal gastrectomy is currently the most widely accepted procedure in the United States, although total gastrectomy is still being evaluated in certain European clinics.

Discouraged by the advanced stage of the disease when it is first diagnosed in the United States, surgeons are using extended lymph node dissections less frequently in this country than in Japan where the concept of "early gastric cancer" was first emphasized and stomach cancer is common.

BILIARY TRACT

The technique of cholecystography, which was first introduced in 1924, allowed an accurate preoperative diagnosis of gallbladder disease (much later, ultrasonography permitted an even more accurate preoperative diagnosis). No longer was it necessary to wait until the inflamed gallbladder could be palpated or the patient became jaundiced before a definitive diagnosis of gallbladder disease could be made. Thus within a few years cholecystectomy became one of the most frequently performed abdominal operations. With the introduction of laparoscopic cholecystectomy, the number of operations being performed has again sharply increased.

Operative cholangiography, which was first introduced in 1931, greatly enhanced the identification of common bile duct stones at operation and lowered the incidence of retained stones to a level that remained fairly constant until the introduction of the choledochoscope.

Less common conditions such as choledochal cysts were rarely diagnosed before middle age, and exploration after repeated attacks of cholangitis presented the surgeon with an inflammatory vascular mass that made any type of surgical approach hazardous whether it involved cyst excision or a bypass procedure such as choledochoduodenostomy or choledochojejunostomy. Recognition of the potential hazard of malignant degeneration of the cyst lining has made excision of the cyst, or at least the mucosal lining, the standard treatment today. Fortunately most of these cases are currently recognized early in life before repeated attacks of cholangitis have caused severe inflammatory changes in and around the wall of the cyst.

Patients with iatrogenic strictures of the bile duct were generally seen late in the course of their disease. They were deeply jaundiced and frequently had biliary fistulas and/or intra-abdominal abscesses. A biliary-duodenal anastomosis, the usual method of repair, was often unsuccessful. The dense hilar scarring that followed repeated unsuccessful repairs led to attempts to establish biliary-enteric drainage via the intrahepatic ductal system, thus avoiding the hilar region. The incidence of strictures declined over the years with the increasing availability of well-trained surgeons to perform the original cholecystectomy, and a hilar Roux-en-Y jejunobiliary reconstruction became an increasingly successful method of repair. A resurgence of ductal injuries has occurred with the rapid introduction of laparoscopic cholecystectomy, which is apparently associated with the so-called "learning curve" of this new technique.

In 1957 several investigators pointed out the difficulties in establishing a differential diagnosis, either clinically or pathologically, between sclerosing cholangitis and carcinoma of the bile duct, and they suggested that certain strictures of the bile ducts, which were thought to be caused by sclerosing cholangitis, might actually be due to a slowly progressing, obscure carcinoma. Today the characteristic cholangiogram of sclerosing cholangitis and its recognized association with other disease (i.e., ulcerative colitis) aids greatly in establishing the diagnosis, although differentiation may, at times, still be difficult.

Intubation, performed either surgically or endoscopically, has been found to provide acceptable palliation if resection of the bile duct carcinoma is not possible. Sclerosing cholangitis may be palliated for years by resection of accessible lesions or by prolonged intubation. Hepatic transplantation is considered in long-standing cases because palliation by other means is often inadequate and there is also the possibility of malignant changes in the bile duct endothelium. A satisfactory "cure rate" for both of these diseases is still being sought.

ESOPHAGUS

Thoracic surgery was in its infancy 60 years ago. The first successful pneumonectomy was performed in 1933, but adequate anesthetic techniques and the dangers of mediastinal infection impeded surgery of the esophagus. The report of a successful intrathoracic esophagogastrectomy for resection of a carci-

noma of the esophagus in 1938 led to increasing attempts to replace part or all of the thoracic esophagus for both benign and malignant lesions by intrathoracic advancement of the stomach or placement of segments of the jejunum or colon through the thorax or in subcutaneous tunnels.

Esophageal reflux was managed largely by nonsurgical means. Resection with gastroesophagectomy was reserved for the most severe cases. The Nissen fundoplication procedure would come later.

The first successful one-stage primary repair of congenital esophageal atresia was reported in 1941 and led to a general awareness of the condition, a more prompt diagnosis, and an increasing number of successful outcomes.

Reports from the Mayo Clinic in 1947 of the successful treatment of bleeding esophageal varices by injection of sclerosing solutions were largely ignored because portacaval shunting, which had been introduced in 1946, seemed more definitive. Only in recent years has the procedure again found a place in the management of acute variceal bleeding.

Because of the risk of life-threatening mediastinitis following resection, patients with cervical esophageal diverticula were frequently not referred for surgical treatment until these diverticula obstructed swallowing because of their large size. The following two-stage operation was suggested: during the first stage the diverticulum was mobilized and elevated in the neck to prevent its filling when the patient swallowed. The wound was then packed to seal the area from the mediastinum. The diverticulum was then excised during the second stage. If the patient was elderly and frail, which often seemed to be the case, and the symptoms were largely relieved simply by elevating the diverticulum, then the excision was omitted.

The Heller operation for achalasia of the esophagus, which had been reported at a meeting of the German Surgical Society as early as 1924, was still in use, but in view of the increasing thoracic surgery experience, attempts were being made to improve results with more extensive operations involving resections. Late results with such procedures, however, have led to a general return to the simpler Heller procedure with the addition of a Belsey Mark IV fundoplication to prevent reflux.

PANCREAS

Acute pancreatitis was usually diagnosed on the basis of clinical findings. If the patient was seen during the day, the diagnosis might or might not be confirmed by means of serum amylase testing. A diagnostic laparotomy was usually required to establish a diagnosis. And even if soap spots and an inflamed pancreas were found, a wedge biopsy of the pancreas was obtained to confirm the diagnosis. Fortunately complications arising from this approach were infrequent.

With the report of a successful two-stage procedure in 1935, interest in pancreatic resection was revived. The "Whipple operation" was followed a few years later by the report of a single-stage procedure not unlike the one most commonly used today to treat various types of periampullary tumors. Although the mortality and morbidity of the procedure are greatly improved, long-term survival figures for pancreatic cancer remain disappointing.

The treatment of chronic pancreatitis is today largely devoted to various procedures that are intended to improve pancreatic duct drainage, with resection reserved for refractory or unusual cases. A wide range of procedures designed to divert bile or gastric juice or both have also been tried, along with injections of both short- and long-acting blocking agents in and around the celiac plexus.

TRAUMA

In the 1930s many surgical residents were trained to treat penetrating injuries of the colon, such as stab or gunshot wounds, by simply primary closure. However, extensive experience with such wounds during World War II, which were typically caused by high-velocity missiles or shrapnel, quickly demonstrated that colostomy or exteriorization was the preferred treatment under combat conditions. However, on returning to civilian life, where injuries were apt to be the result of small caliber missile trauma or stab wounds and where prompt treatment was generally available, surgeons resumed their frequent use of primary closure.

Preparation of the colon for an elective operation consisted primarily of measures designed to promote mechanical cleansing of the colon and included several days of a liquid diet along with a laxative followed by cleansing enemas. Techniques and instruments were devised to enable the surgeon to perform a so-called "closed" anastomosis following segmental resections. With the patient well prepared and the procedure properly performed, the results were remarkably good. As a matter of fact, some of the more experienced colon surgeons temporarily returned to the cleansing procedure after encountering some of the complications that accompanied the initial attempts to "sterilize" the colon with large doses of the early broad-spectrum antibiotics.

Cases of diverticulitis severe enough to warrant

surgical management were treated with a three-stage procedure comprising (1) proximal colostomy, (2) resection of the diseased segment, and (3) finally, closure of the colostomy. Even after the advantages of the one-stage or, at times, the two-stage procedure had been demonstrated, the three-stage technique continued to be widely used for some time.

Until 1948, when the importance of the aganglionic rectal segment was recognized as the causative factor in most cases of megacolon in pediatric patients, many of these cases were treated with colostomy or partial resection of the distended proximal colon. Since the acceptance of the basic concept of the aganglionic segment, a variety of procedures have been introduced in an attempt to enhance the safety of the operation and improve postoperative bowel function.

POSTSCRIPT

Although there have been remarkable refinements in diagnostic procedures and operative techniques over the past 60 years, including the more recent introduction of endoscopic and laparoscopic procedures, the contrast between results of present-day treatments and those achieved more than a half-century ago can be attributed primarily to the following factors: a better understanding and improved management of surgical or traumatic shock, a greater understanding of the metabolic response to injury, remarkable advances in anesthetic support with widespread adoption of endotracheal anesthesia and use of curare-like agents, and the development of powerful antibiotics starting with the introduction of penicillin. Further refinements and improvements of these basic contributions are ongoing.

State-of-the-Art Address

Fighting in the Fortress of Medicine: Surgical Competence in the Era of Managed Care

Albert R. Jonsen, Ph.D.

I am pleased and honored to be giving this year's State-of-the-Art Address. You can anticipate a rather different type of lecture since I am a philosopher, not a surgeon, and I know little of your science and art except from the standpoint of having been a surgical patient. I will be speaking on the generic topic of medical and surgical competence, in particular the ethical imperative of competence in the era of managed care. Even more specifically, I will be discussing the era of managed care, which is upon us and is our future. I will talk about this very modern problem by first filling you in on some medieval medical history. This unfamiliar episode in the history of medicine has, in my opinion, amazing relevance to the modern problems faced by medicine today.

Nine hundred years ago, several Italian merchants felt called upon to serve God in some charitable work. They decided to dedicate their lives to serving the sick pilgrims in the Holy Land and so, only a decade before the Crusades began, they opened a hospital in Jerusalem. They called themselves the Brethren of the Hospital of St. John of Jerusalem. Within a few years they had opened hospitals elsewhere along the pilgrim routes.

The Hospitals of the Brethren spread from Jerusalem through the Near East and into Europe. They were the prototype of the hospital as we know it today. A strict regimen was observed. Physicians were required to visit each patient twice a day and to record the patient's progress on a chart hung on the bed. Grand rounds were held weekly and autopsies were performed once a month. Prescriptions were written and filled by a pharmacy. Each patient's food was prepared in conformity with his or her illness. All who were sick and in need of medical attention were admitted regardless of whether they were Christian, Muslim, or Jewish. Above all, the Brethren, as physicians, nurses, and administrators, acknowledged that they had obligations to their patients. The rules of the hospital had an overriding imperative: "The Brethren of the Hospital should serve our lords, the sick, with zeal and devotion as if they were vassals to their Lords" (Rule of 1181). In other words, they viewed those whom they served as having authority over them, just as the medieval lord had authority over his vassals and serfs. Never before in the history of medicine, even in the noble Hippocratic texts, had there been such an explicit acknowledgment of a duty toward the sick.

However, the Brethren were caught in the Crusades. They realized that their hospitals were vulnerable and they quickly established a security force that grew into a military arm. They changed their name from Brethren of the Hospital to the Knights of the Hospital. Although the hospital work continued, the military mission expanded. Every member was required to serve half a year in each branch of service. Within a century they had become the largest single military and naval force of Christendom. Their hospitals had become fighting fortresses.

Historical records show that from the mid-twelfth century onward, the hospital administrators constantly complained to headquarters that funds which should have been devoted to the care of the sick were being diverted to the military units. They built great castles. One of these, Crac de Chevalier, near Damascus, Syria, was described by Lawrence of Arabia as "the most wholly admirable castle in the world." When French archeologists published their study of that massive fortress in the 1930s, they said, "It is not possible to say with certainty where the infirmary was." A work that had started with caring charity toward the sick poor had been transformed into something quite different—a vast complex engine of military might within which, somewhere hidden and obscure, the work of healing went on.

This story from the medical past carries an obvious message for the medical present. Modern medicine

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has been built into a fortress. Over the past 50 years the structures of science, finance, insurance, government, and industry have been added as the walls, towers, bastions, and moats around the work of healing. This has become necessary for the following reasons: expanding skills and advances in medical technology require ample and complex support; capital must be raised to finance buildings; insurance is needed to pay for medical care; and government and business investments are needed to fund research and provide loans for education. Modern medicine would not be modern were it not surrounded by all of these structures. Yet we are coming to see, as never before, that each of these structures makes its own stringent demands and each has its own purposes and values, and the more intricately involved medicine becomes with these demands, purposes, and values, the greater the impact that will be felt by medicine. Indeed, not a few practitioners of medicine have accepted these demands, purposes, and values as their own, under the oft-heard maxim, "well, after all, medicine is a business."

Might the time not come when our great modern fortress of science and medicine will be as empty and desolate as the Crac de Chevalier? Not in the physical sense but empty in the spiritual sense, for the spirit of healing and caring for our lords, the sick, will be gone. We might then ask—indeed, we may ask now, "where is the infirmary?" You have all been touched by the encroaching demands of foreign powers on your practices. I do not need to relate these problems in detail, which include constrained reimbursement, "incentivized" salaries, restricted referrals, close oversight, conflicting contracts, a plethora of paperwork, diminishing resources for teaching and research, and shifting populations of patients. Complaints concerning all of these difficulties have become daily occurrences within the profession. Exactly how all of this turmoil and all of these techniques will affect quality of care or patient satisfaction or health outcomes or costs remains uncertain. Almost the only thing we do know for sure is that the salaries of CEOs keep escalating. Amid the complaints and queries, we must continue to ask, "where is the 'infirmary,' that is, where in this new business are the sick being cared for?"

Of course, patients are still present in large numbers and most physicians and surgeons still devote most of their time and energy to caring for them. Obviously, in the cavernous and complex buildings in which we work, there are surgical suites and patient beds. That is not the point, however; the question remains, is there something essential about patient care that is being overwhelmed by the systems of money, management, and law that we call health care? Not a few doctors and patients suspect there is.

I suggest that one essential element of care that is endangered is medical and surgical competence. I will attempt to explain my suggestion. Dr. Lawrence Way, in his Presidential Address to The Society last year,[1] referred to my philosophy of competence. He quoted from my book entitled *The New Medicine and the Old Ethics*,[2] "The cardinal ethic of medicine is competence: The possession and use of the requisite knowledge, technical skill, and humanism. The relationship between the public and the medical profession hinges on trust, for the public is obliged to depend on the claims backed by certification that the professionals are competent to care for its problems." Of course, the idea that the cardinal ethic of medicine is competence is not mine originally. It was enunciated by Hippocrates, whose axiom, "benefit the patient and do no harm," clearly imposes a duty to know what interventions will be beneficial and under what circumstances they should be used. The common assertion that Hippocrates was the father of Western medicine arises from the spirit of investigation into the course of disease and the effects of treatment that he fostered, a spirit of investigation motivated by the ethical imperative to benefit the patient and do no harm.

It took more than two millennia for that spirit to be converted into the science of medicine as we know it today, based on an empirical anatomy, physiology, and epidemiology. During the intervening centuries, when the practice of medicine was carefully monitored by the Catholic church, physicians and surgeons were taught that they would incur the penalties of mortal sin if they practiced without requisite knowledge and experience. That knowledge was found in the books of Hippocrates, Galen, Avecinna, and Maimonides. That experience was gleaned from the collected observations of practitioners and was often random and unverified. During the nineteenth century, knowledge moved from philosophical speculation to a juxtaposition with systematized, verified observation, and thus modern medicine and surgery came into being. It was at the end of the nineteenth century and the beginning of the twentieth that Richard Cabot, Professor of Medicine at Harvard University, began to emphasize competence as the cardinal ethic. Chester Burns,[3] an historian of medicine, writes of Cabot:

> By the fourth decade of the 20th century, the American medical conscience had been reshaped. The all-purpose general practitioner of the 19th—a Christian gentleman of intrinsic goodness, law abiding and loyal to the codified roles of professional society—had been replaced by a new group of specialist practitioners... rooted in experimental science and elaborate methods of clinical evaluation and patterns of clinical care...What counted for Dr. Cabot was whether a practitioner understood specific diseases, their

causes, signs, symptoms, courses, prognoses, treatments—and whether each practitioner applied this understanding in the assessment and management of each individual patient.

We have come to take Cabot's view for granted. This is merely good medicine. We have come to use the term "ethics" for another set of issues—that is, whether patients are informed of their options and choose their own treatment, whether confidentiality is kept, and whether providers are compassionate and humane. But in Cabot's view, however important these features are—and he considered them very important—they are secondary to the cardinal ethical duty, which is competence. I describe this as the ethic of Cabotean competence. Dr. Way's Presidential Address was subtitled "Technology and Competence," and he spoke of the duty to master the emerging technologies of surgery and of the problems faced by training programs in imparting sufficient knowledge, skill, and experience to fulfill the public pledge of competence that certification holds out to the public.

Today we are at a frontier where Cabot's ethic of competence, now a century old, needs to be reevaluated and perhaps even expanded. We are all only too well aware of the rapid, extensive shifts in the financial, organizational, and managerial aspects of health care. We are all familiar with the dramatic scientific and technologic advances, ranging from molecular biology to cybernetics, that are speeding medical and surgical knowledge into the twenty-first century. We now know that the devices which allow for direct visualization and lessen the degree of invasiveness have and are continuing to transform surgery at its very core. Cabotean competence would call for an ever-increasing mastery of these advanced technologic and scientific skills, used to their highest and best capacity, regardless of the financial, organizational, and managerial system in which the surgeon works. (Actually Cabot was quite sensitive to these aspects, but the concept of competence that we inherit from him sets them aside.) Indeed, in actual fact, today's surgeons are probably the most technically competent doctors in history. Yet we all know how problematic it is to competently utilize the emerging technologies within these new systems. The systems have changed the flow of patients, controlled acquisition of, and access to, new technology, and reshaped training and research opportunities—sometimes in manifest but other times in subtle ways. How is Cabotean competence possible in the new world of health care?

I will not describe this new world to you. You who must work in it know it better than I. I will not attempt to dictate how the new surgical science and modalities will fit into that new world; you are the ones who must discover that. I will only propose an ethic, a revision or expansion of the Cabotean ethic of competence, that I believe must guide the movement of surgical practice into the new structures of health care. An ethic is not a plan of action; rather it is a hope that guides the search for the best action among alternatives—a hope by which those who search oblige themselves not to settle for merely satisfying their self-interests.

The expansion of Cabotean competence that I envision might best be explained by a brief excursion into etymology. The word "competence" comes from the Latin verb, "competere," which means "to compete," as in a race or a game. The competent person is one who can keep up, who has the ability to run the course from start to finish. Our modern notion of competence has lost some of this competitive sense, but it can still be felt in the intense demands of training and in the stress of certification examinations. However, the Latin word has a deeper meaning; it literally means "to seek together." Those who compete in a race all seek the gold, not cooperatively of course, but all must run together on the same track. Now, seeking together can also mean collaboration, wherein several parties bring their respective energy and skill to the task. I maintain that the surgical competence of the future must be expanded from individual mastery of technique to collaborative development and control of the modalities proper to surgery. The ethic beyond Cabotean competence is collaborative care.

Medical and surgical competence has always been essentially an individual virtue. Each surgeon must master the intellectual moves needed to plan an operation—that is, the physical maneuvers necessary to excise a tumor, transplant an organ, revise anatomic structure, or incise and suture—and each must also safely manage the postoperative course. Each surgeon is judged more or less competent in each of the phases of surgical work. Furthermore, not only is competence an individual virtue, but surgical practice is essentially an individual activity. Certainly surgeons consult with one another concerning their cases; several surgeons can collaborate on a single case and the outcomes of cases can be collectively criticized. Still, surgery, like almost all of modern medicine, is the work of individual physicians responsible for their own patients. In general, American medicine has not been a collaborative enterprise. Certain enclaves, such as university departments, research teams, and long-established staff model HMOs, have developed collaborative conventions, but for the most part physicians and surgeons work alone.

The ethic of collaboration must succeed or supplement the ethic of competence. Unquestionably, many

of the emerging financial, organizational, and managerial innovations in health care are forcing physicians and surgeons into collectivities of one sort or another, corralling practitioners into foreign and often onerous, odious arrangements. Also, some practitioners are discovering ways of cooperating for their own profit. I am not speaking of these factual forms of collaboration. Rather, my collaboration is an ethic—that is, a hope of finding ways to benefit patients without causing them harm within the new structures of finance, organization, and management.

This collaboration must take several forms. The first, and most obvious, is one that is already occurring, namely, the collection and analysis of surgical outcomes in the most sophisticated manner and the use of that analysis to distinguish more clearly than ever before the effective from the ineffective, the promising from the deceptive. Much work is being done on this form of collaboration, to define outcomes more clearly and fully, to collect requisite data, and to disseminate results. Much more needs to be done to encourage innovators, who are always eager to push ahead, to enter the slower, more precise world of technology assessment. A similar form of collaboration, the systems of surveillance and prevention of surgical error and iatrogenic complications, is also becoming familiar in the surgical world and must continue to be perfected.

Beyond these joint efforts, other much more difficult and unfamiliar collaborations must be effected. The constant interchange of information and technique between surgical subspecialists and general surgeons, and between surgeons and other specialties, is an ethical imperative that must fight against long-ingrained insularity and self-interest. The specialties and subspecialties of American medicine have, for a century, been continents of independent nation states, each founded on the biologic constitution of an organ system and possessing an armory of its own inventions. Unquestionably, specialization has advanced science and practice. Still, its progress has been won by battles over new inventions and new "nations" have arisen, such as cardiothoracic surgery and interventional radiology, to take possession of the organ system of an older nation. The growing realization that technology transcends limited uses and can be molded to suit many purposes requires a new mentality, not only a truce but a common market and a union. The collaboration of practitioners and researchers from all segments is demanded not only by the constant deconstruction of artificial barriers between the sciences but for the benefit of patients whose conditions can be understood and managed only with a variety of skills and tools. The undesirable consequences of the new managerial technique of "gatekeeping" will ultimately be avoided only when the so-called gatekeeper is consciously working within a collaborative environment of specialists. Patients whose conditions are a pathophysiologic complex capable of being understood and managed by several specialists must be considered as a single patient to be managed by a unified, collaborative, mutually supportive set of providers rather than being passed back and forth between rivals, as so often occurs today.

A third form of collaboration must link physicians and surgeons from all specialties across the walls and moats of the myriad corporations that now establish and maintain the financial, organizational, and managerial aspects of health care. These corporations, by their very nature, will seek to weld their practitioners' loyalty to their corporate goals. That is the corporate ethic and it will invade the professional ethic. There must be a transcorporational ethic of collaboration. All specialties, along with each of their own professional associations, must work to articulate and enforce standards of care and of training that the corporations will honor. We have barely begun to find the means for this transcorporate collaboration of professions. Still, there cannot be differing standards of care and competence under different corporate flags.

Behind all of these collaborations dwells the realization that you, the physicians and surgeons, are the essential resource in health care. We have become so familiar with the phrase "delivery of health care" that we have almost forgotten that what is being delivered is you. Your diagnostic and therapeutic skills brought to the patient in real time is what health care is at its essence. The managers can set up clinics, provide information, buy practices, and bill patients, but they cannot deliver care. You can and they must use you. My ethic of competence and collaboration says that you must allow yourselves to be "delivered" only as instruments of medicine and surgery of quality and only as agents of an ethic of service to the sick. There are dangers in these sorts of collaborations. Collaboration can become monopoly. The "watchdogs" of the Federal Trade Commission will often bark, smelling money even beneath ethics. Still, there is a long and powerful tradition of professional autonomy in technical matters that can and must be continually asserted. Its assertion requires, however, that even that autonomy be executed within the collaborative activities of clinical studies and technology assessment.

This, then, is the ethic of collaboration that must complement the ethic of competence. I am not simply urging that technically competent surgeons communicate better with one another and with various other persons. I am suggesting that the very definition of surgical competence be expanded to include collaboration—that is, surgeons must not only attain the in-

tellectual and manual skills for their tasks, but they must also build around themselves the collaborative activities that will make possible the most efficient execution of those tasks in the new environments. I have no doubt that economic and political forces will make this difficult to achieve; I have no doubt that the ingrained insularity of the specialties will make it slow to come about, and I have no doubt that the intrinsic difficulty of knowing when collaboration will be fruitful will make even the most committed persons hesitate. Finally, I imagine that many view these proposals as unrealistic and unrealizable. Still, it seems to me that unless competence is expanded into competent collaboration, the most ancient and honorable ethic of medicine and surgery—that the sick be helped and not harmed—will be lost in the complex evolving world of science and technology, finance, and business and management.

In modern medicine the care of the patient takes place within our great fortresses of science and medicine. There are bastions of business, moats of management, walls and gates of law and regulation that surround the care of the patient, and they are being amplified and fortified by the day. Perhaps the time may come when our great fortress of science and medicine will be as empty and desolate as the Crac de Chevalier—not so much in the physical sense but empty in the spiritual sense, for the spirit of healing and caring for our "lords," the sick, will be gone. We might then ask—indeed, we may ask now—"where is the infirmary?" The answer is and always has been, in Hippocratic times, in medieval medicine, and today, the infirmary is where competent medicine is practiced and where the doors are open to the sick. And, in our times, competent, accessible medicine can exist only when collaboration is an ethical imperative.

REFERENCES

1. Way L. General surgery in evolution: Technology and competence. Am J Surg 1996;171:2-9.
2. Jonsen AR. The New Medicine and the Old Ethics. Cambridge: Harvard University Press, 1991.
3. Burns C. Richard Cabot and reformation in American medical ethics. Bull Hist Med 1977;51:353-368.

Diagnostic Value of CA 19-9 in Patients With Pancreatic Cancer and Nonspecific Gastrointestinal Symptoms

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Serum expression of the tumor marker CA 19-9 was studied in 2119 patients. The discriminating capacity between benign and malignant disease was high for CA 19-9, especially in patients with pancreatic cancer (n = 347).The sensitivity of CA 19-9 was 85%. In patients who were Lewis blood type positive, the sensitivity increased to 92%. CA 19-9 levels were significantly lower in patients with resectable tumors (n = 126) than in those with unresectable tumors (n = 221, $P < 0.0001$; sensitivity 74% vs. 90%). CA 19-9 levels dropped sharply after resection but normalized in only 29%, 13%, and 10% of patients with stage I, II, and III tumors, respectively. In unresectable tumors no significant decrease in CA 19-9 levels after laparotomy or bypass surgery was found. Among patients with the same tumor stage, the median survival time in those whose CA 19-9 levels returned to normal after resection was significantly longer than in those who had postoperative CA 19-9 levels that decreased but did not return to normal (stage I, 33 months vs. 11.3 months; stage II, 41 months vs. 8.6 months; and stage III, 28 months vs. 10.8 months). In patients with recurrent disease, 88% had an obvious increase in CA 19-9 levels. CA 19-9 measurement is a simple test that can be used for diagnosis, for evaluation of resectability, and for prediction of survival after surgery and recurrences. (J GASTROINTEST SURG 1997;106-112.)

The incidence of pancreatic cancer has increased during the past three decades. The clinical picture of pancreatic cancer can mimic a number of other nonmalignant gastrointestinal diseases. Because there are no specific symptoms, especially in the early stage, only about 5% to 10% of pancreatic cancers can be resected curatively.[1]

Monoclonal antibody 1116-NS-19-9 (19-9 Ab) was obtained after immunization of mice with human colorectal adenocarcinoma cell lines.[2] The antibody reacts with sialyl-lacto-N-fuopentaose, corresponding to sialylated blood group antigen Lewis^a.[3,4] Elevated serum CA 19-9 levels are found in a high proportion of patients with gastrointestinal malignancies, particularly in those with pancreatic cancer.[5] The purpose of this investigation was to determine the sensitivity and specificity of the assay in diagnosing cancer of the pancreas, to predict resectability rates, and to examine the utility of determining serum CA 19-9 levels as a prognostic indicator and as a test for recurrent disease in order to monitor patients who have undergone treatment of pancreatic carcinoma.

STUDY GROUPS

Patients

Cancer of the Pancreas. A total of 347 consecutive patients with biopsy-proved adenocarcinoma of the pancreas, referred to the First Surgical Clinic of Ulm University, served as the study group. Serum was obtained from these patients on postoperative days 1, 3, 6, 9, and 12, before any definitive therapy was instituted, and monthly thereafter in patients with resectable tumors only. Resection of the carcinoma was performed in 126 patients; of these patients, 45, 10, 58, and 13 showed stage I, II, III, and IV disease, respectively (TNM classification).[6] In the patients with resected adenocarcinoma, additional follow-up stud-

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ies included routine clinical examination with chemical and pharmacologic profiles and CT scan 3 months after the operation. If the tumor marker CA 19-9 was found to be increased on follow-up, a search for a metastatic source was conducted independent of the follow-up procedures.

Control Subjects

Benign Pancreatic Diseases. On admission to the hospital, serum was obtained from 300 patients with chronic alcoholic pancreatitis (diagnosed on the basis of endoscopic retrograde cholangiopancreatography, CT scan, and laboratory and pancreas function tests). All of these patients were followed up after they had received the appropriate therapy for 6 to 12 months.

Nonpancreatic Benign Disorders and Healthy Persons. Serum from 786 patients with benign diseases treated surgically was obtained for determination of CA 19-9 values. Among these patients 55 had bilirubin levels greater than 2 mg/dl. The diagnosis of benign jaundice was based on a review of their hospital records after discharge. Serum was also obtained from 691 patients with extrapancreatic malignant diseases (i.e., gastrointestinal malignancies not including the pancreas).

METHODS

Assay

Serum samples were stored at −20° to −70° C, and these specimens were then assayed within 1 week. The CA 19-9 concentration was determined by a solid phase radioimmunoassay (Centocor, Inc., Malvern, Pa.). A value of 37 U/ml was used as the upper limit of normal. One unit of CA 19-9 antigen corresponds to 0.8 ng/ml of pure antigen.[7] Serum carcinoembryonic antigen (CEA) values in patients with pancreatic cancer were estimated by means of an enzyme immunoassay (Isotopen Diagnostic Dreieich, Federal Republic of Germany) using a cutoff value for CEA of 3 ng/ml.

Statistics

Actual survival curves were constructed using the methods of Kaplan and Meier,[8] and statistical comparisons were performed according to the Mantel-Haensel procedure.[9] Determinations of the statistical significance of comparisons of ratios were calculated using the chi-square test.[10]

Sensitivity was defined as the number of patients with cancer of the pancreas with an elevated assay divided by the total number of patients with cancer of the pancreas. Specificity was defined as the number of control subjects with a nonelevated assay divided by the total number of control subjects.

RESULTS

Diagnostic Value of CA 19-9

The sensitivity of CA 19-9 in patients with pancreatic carcinoma and benign diseases is shown in Table I. An elevated CA 19-9 level was found in 296 (86%) of the 347 patients with pancreatic carcinoma. The median assay value of these patients was 288 U/ml (range 6.9 to 70.000 U/ml). Elevated CEA values were found in 64% of the patients. The difference in the sensitivity of CA 19-9 and CEA was significant ($P < 0.0001$). By taking a CA 19-9 normal limit value of 37 U/ml, it was possible to determine a specificity of 87%.

Simultaneous estimation of serum CA 19-9 and bilirubin concentrations was conducted preoperatively in 249 patients with pancreatic carcinoma; 121 patients showed jaundice preoperatively. It is interesting to note that the sensitivity of CA 19-9 was unaffected by serum bilirubin levels (Table II). Twenty-four (38%) of the additional 55 patients with benign jaundice had elevated CA 19-9 levels (false positive value).

Correlation Between CA 19-9 Level and Tumor Stage or Resectability

The sensitivity of CA 19-9 in patients with resected pancreatic carcinoma (n = 126) was 77% and in patients with unresectable pancreatic carcinoma, 90%

Table I. CA 19-9 assay values in pancreatic carcinoma and control groups

CA 19-9 (U/ml)	Pancreatic carcinoma		Chronic pancreatitis		Extrapancreatic malignancies		Benign disease	
	No.	%	No.	%	No.	%	No.	%
6-37	51	14	252	14	488	71	699	89
38-120	54	16	40	13	100	14	81	10
>120	242	70	8	3	103	15	6	1
TOTAL	347		300		691		786	

Table II. Relationship between preoperative CA 19-9 and serum bilirubin levels

Serum bilirubin level	No.	Preoperative CA 19-9 levels >37 U/ml No.	%	>120 U/ml No.	%	Median (range)
Normal	108	93	87	73	68	405 (7-28,900)
Elevated	121	104	86	90	74	503 (7-20,000)

Table III. Sensitivity of CA 19-9 and CEA in pancreatic carcinoma

Tumor	CA 19-9 (>37 U/ml) No.	%	Median (range)	CEA (>3 ng/ml) No.	%	Median (range)
Stage I (n = 45)	29	64	68 (9.0-3.018)	22	49	2.5 (0.7-26.5)
Stage II (n = 10)	8	80	72 (8.4-5.000)	5	50	2.5 (1.0-12.6)
Stage III (n = 58)	47	81	210 (2-7.496)	29	50	2.9 (1.1-1.500)
Stage IV (n = 13)	13	100	412 (49.6-14.600)	9	69	5.7 (0.6-40.8)
Resectable (n = 126)	97	77	152 (2-14.600)	68	54	3.4 (0.6-1.500)
Local inoperable (n = 104)	93	89	387 (7.3-20.000)	65	62	4.6 (1.4-341)
Local inoperable and distant metastases (n = 117)	106	90	670 (2-70.000)	89	76	6.8 (0.7-1.011)
Nonresectable (n = 221)	199	90	512 (2-70.000)	166	75	5.8 (0.7-1.011)

CE = carcinoembryonic antigen.

(n = 221). The median serum CA 19-9 levels for these two groups were 152 U/ml (range 6.9 to 14.600 U/ml) and 512 U/ml (range 6.9 to 17.000 U/ml), respectively. The difference in sensitivity was statistically significant ($P < 0.01$) (Table III). Elevated serum CEA levels were found in 54% of patients with resectable and in 75% of those with nonresectable tumors. Median CEA values for these two were 3.4 ng/ml (range 0.6 to 1.500 ng/ml) and 5.8 ng/ml (range 0.7 to 1.011 ng/ml), respectively. The CA 19-9 value in the 126 patients with resected adenocarcinoma of the pancreas was estimated in accordance with TNM tumor stages. More patients showed elevation of CEA and CA 19-9-serum levels if the tumor stage was increased (Table III).

Resectability of Pancreatic Cancer Depending on Pretreatment Serum CA 19-9 Level

Pretreatment values ranged from greater than 37 to less than 400 U/ml in 139 patients; 41% had resectable disease. The pretreatment CA 19-9 values exceeded 400 U/ml in 157 patients and only 18% had resectable disease ($P \leq 0.0001$). Table IV shows the resectability rate depending on the preoperative CA 19-9 value; 68% of patients with resectable disease showed preoperative values less than 400 U/ml and 32% of the same patients had values greater than 400 U/ml. Among patients with nonresectable disease or distant metastases, 39% had CA 19-9 values less than 400 U/ml and 61% had values greater than 400 U/ml ($P < 0.0001$).

Response to Tumor Resection: Prediction of Survival

One hundred twenty-six out of 347 patients underwent a standard resection including 13 patients who also had a partial hepatectomy (stage IV disease). Among the 113 patients with stage I, II, and III disease, 84 of them showed preoperative pathologic CA 19-9 values. In only 14 of the 84 patients who underwent surgical resection of the carcinoma (9 in tumor stage I, 1 in tumor stage II, and 4 in tumor stage III) did the CA 19-9 levels fall to within the normal range. Among patients in the same tumor stage, in whom CA 19-9 levels had returned to normal, CA 19-9 levels after definitive treatment were associated with a longer period of survival than those with CA 19-9

Table IV. Resectability in pancreatic cancer (pretreatment CA 19-9 serum levels)

	Serum levels of CA 19-9 (U/ml)													
	<120	>120	<200	>200	<300	>300	<400	>400	<500	>500	<600	>600	<700	>700
Resectable disease	44%	25%	43%	23%	41%	21%	41%	18%	39%	17%	38%	16%	37%	16%
Nonresectable tumor or with distant metastases	56%	75%	57%	77%	59%	79%	59%	82%	61%	83%	62%	84%	63%	84%

Table V. Survival depending on postoperative CA 19-9 values in patients with resectable tumors (stages I, II, and III)

Tumor stage	Postoperative CA 19-9 (U/ml)	No.	Median survival time (mo)	*P* value
I	>37	20	11.3	0.003
	<37*	9	32.9	
II	>37	7	8.6	0.1
	<37*	1	41	
III	>37	43	10.8	0.03
	<37*	4	28.2	
TOTAL (I, II, and III)	>37	70	10.9	0.0001
	<37*	14	32.2	

*Normalization of CA 19-9 serum levels as found 4 weeks (median) postoperatively (range 1 to 13 weeks).

Table VI. Survival depending on preoperative CA 19-9 levels in patients with resected carcinomas

Preoperative CA 19-9 level (U/ml)	No.	Postoperative survival time (mo)		*P* value
		Median	Range	
<400	89	17.3	6-104	0.0001
>400	37	7.1	6-25	
<400	61*	18.1	7-60	0.001
>400	35*	7.1	6-25	

*Only patients with preoperative CA 19-9 values >37 U/ml.

levels that never returned to normal (Table V; $P < 0.05$). In patients with unresectable tumors (n = 221) and those with stage IV disease (n = 13), no significant changes in CA 19-9 levels were found after surgery.

Pretreatment Serum CA 19-9 Levels in Patients With Resectable Disease Correlated With Survival

Of the 126 patients with resectable adenocarcinoma (stages I to IV), 89 had preoperative values less than 400 U/ml (group I) and 37 patients had values greater than 400 U/ml (group II). The median survival time was 17.3 and 7.1 months in groups I and II, respectively (Table VI).

Response of CA 19-9 to Recurrence or Metastasis

One hundred sixteen of the 126 patients were allocated to a follow-up program. In 113 of these 116 patients, distant metastases or local recurrences subsequently appeared within the median follow-up time of 12 to 28 weeks (Table VII). A secondary elevation of the CA 19-9 level was detected in 90% of the patients who had recurrences. Secondary elevation of the tumor marker preceded changes that were de-

Table VII. Recurrence of pancreatic carcinoma and CA 19-9 values

Tumor stage	Recurrence		No follow-up	Recurrence free	Diagnosis of recurrence		CA 19-9 increase		Lead time of CA 19-9	
	No.	%	(No.)	(No.)	Wk postop	Range	No.	%	Wk	Range
I (n = 45)	41/42	98	3	1	28	8-168	29/33*	88	20	6-56
II (n = 10)	8/9	89	1	1	28	8-44	5/5*	100	16	7-44
III (n = 58)	51/52	98	6	1	24	4-64	37/42*	88	4	9-49
IV (n = 13)	13/13	100	0	0	12	4-20	10/10	100	5	1-10
TOTAL (n = 126)	113/116	97	10	3			81/90	89		

*In eight, three, and nine patients with stage I, II, and III tumors, respectively, and an additional three patients, CA 19-9 values were not measured at the time of diagnosis of recurrence.

tectable on CT scan or clinical examination by a median time ranging from 4 to 20 weeks (Table VII).

DISCUSSION

The diagnosis of cancer of the pancreas can be expensive and invasive. Accurate and efficient diagnosis of pancreatic cancer continues to be a difficult clinical challenge. Although various diagnostic imaging tools may be useful, all are limited in their specificity and sensitivity, and they involve considerable expense. Recent studies[4,11] have shown that pancreatic cancer is associated with the appearance of glycoconjugates bearing a sialylated derivative of a Lewis (Le) blood group determinant. Studies confirm that neither the Le^a antigen nor CA 19-9 can be synthesized in $Le^{(a-b-)}$ subjects because of the lack of fucosytransferase.[12] A sensitive and specific marker of pancreatic carcinoma may be used as follows: (1) for diagnostic screening of high-risk patient populations with nonspecific signs or symptoms, possibly resulting from pancreatic carcinoma; (2) as an aid in deciding whether or not to resect the pancreas in patients with a pancreatic mass, if a tissue diagnosis cannot be made before resection; or (3) as an aid in evaluating the efficacy of a particular treatment, if the marker level reflects overall tumor burden. Additionally, if high levels correlate with large tumor burdens, assay of a serum marker may aid in selecting of patients with pancreatic cancer who may be candidates for surgical exploration.

In clinical studies the accuracy of ribonuclease, ferritin, lactoferrin, various gastrointestinal hormones, and pancreatic oncofetal antigens, as well as the alpha-fetoprotein, have been evaluated in the diagnosis of pancreatic carcinoma.[13,14] Thus far, none of the above-mentioned markers has proved to be specific for pancreatic carcinoma.[5] CEA levels have been studied, but there have been many reports of problems with the sensitivity and specificity of the CEA assay in detecting cancer of the pancreas.[15] Using a reference cutoff of 3 ng/ml for CEA, sensitivities of 36% to 80% and specificities of 58% to 95% have been reported. We have confirmed these difficulties with CEA in this study, showing that the sensitivity of CEA at 3 ng/ml is 64% and that the difference in the sensitivity between the two markers, CEA and CA 19-9, is statistically significant ($P < 0.05$).

Another possible serum marker for pancreatic carcinoma, DU-PAN-2, seems to be promising.[16] Elevated serum levels have been found in nearly 95% of patients with pancreatic carcinoma who have been tested. In another report sensitivities of 64%, 68%, and 84% were found.[17-19] The *ras* gene mutation was found in peripheral blood in two of six patients,[20] in 7 (70%) of 10 frozen pancreatic tissue specimens,[21] in seven of nine pancreatic cancer tissue specimens,[22] and in 67% of pancreatic juice specimens,[23] and in stool specimens from 6 of 11 patients with pancreatic adenocarcinoma. Until now the CA 19-9 assay appears to have shown more promise as a simple diagnostic test than the above-mentioned markers. Numerous investigators have reported on the utility of CA 19-9 in early and differential diagnosis of pancreatic adenocarcinoma. The sensitivity (69% to 93%) and specificity (78% to 98%) reported in our clinic and various series were quite encouraging.[24] The difference in sensitivity in various publications is due to the fact that patients with pancreatic cancer vary from one series to another with regard to tumor stage (i.e., a series with more patients in advanced stages shows a higher sensitivity than a series with more patients in early stages). We would, therefore, advocate the use of an internationally uniform cutoff value; furthermore, the sensitivity should always be reported as it relates to tumor stage. The sensitivity of the CA 19-9 assay was not dependent on clinical signs (especially in patients with jaundice) and symptoms; it was only dependent on tumor mass and stage.

The CA 19-9 serum level is elevated not only in patients with pancreatic cancer, but also in patients with extrapancreatic malignancies and in some pa-

tients with benign diseases. Therefore the assay for CA 19-9 is neither 100% sensitive, especially for early tumor stage, nor specific for pancreatic cancer.[25]

One of the important factors limiting the sensitivity of the serum CA 19-9 assay is that subjects with the Le blood group hardly ever produce CA 19-9 antigen.[26] In the Le-positive group, 92% of patients with pancreatic cancer showed pathologic levels of CA 19-9 preoperatively and in 95% of patients with recurrences after resection of the carcinoma, elevated levels of the marker were found.

The current study was undertaken to examine the utility of the CA 19-9 assay level correlated with resectability, prognosis, and detection of recurrences. Our data for the 347 patients indicate that higher levels of serum CA 19-9 correlated with larger tumor size and advanced tumor stage, even though much overlap existed. Median pretreatment values for CA 19-9 were found to increase from stage I to stage IV and from resectable to nonresectable tumors. This supports the concept that CA 19-9 levels can be used as an indicator of tumor burden. Also, relatively high pretreatment levels of CA 19-9 (300 U/ml or more) indicate that 80% of the carcinomas are not resectable. Although only slightly elevated, CA 19-9 levels can reflect a better prognosis, even in resectable carcinomas (median survival time in patients with a CA 19-9 level of less than 400 U/ml was 17.3 months; for levels greater than 400 U/ml, the median survival was 7.1 months).

Such slightly elevated values require that other diagnostic means be used to rule out nonmalignant diseases of the pancreas or biliary tract. Our data also show that the higher the level of CA 19-9, the greater its specificity. In addition, a high CA 19-9 level suggests not only the nonresectability of the tumor but also, in patients with resectable disease, a poor prognosis and short postoperative survival time (Table VI).

In our opinion the treatment of choice in the follow-up of patients with pancreatic cancer is monthly measurement of tumor markers plus clinical examination. An imaging procedure should not be used initially. In patients who have not undergone resection, tracking the marker values may help to assess the effectiveness of systemic or radiation therapy. In patients who have had resections, the marker pattern may initially predict the prognosis and later on may signal a relapse. An imaging procedure should be used only if symptoms appear and if the marker values rise or if a relapse is suspected.

Based on our observation of 84 resectable patients with pathologic preoperative CA 19-9 levels, a comparison of preoperative and postoperative serum CA 19-9 levels could provide information about the effectiveness of the operative resection. Each of the 84 patients who had their tumors removed had a sharp decrease in the serum concentration of CA 19-9. Postoperative normalization of the marker values was found in 9 of 29 patients with stage I tumors, in one of eight with stage II tumors, and in 4 of 47 with stage III tumors. Only in the 16.6% (14 of 84) of resectable patients with preoperatively pathologic CA 19-9 values, that is, in 4.7% of the entire group of patients with pancreatic cancer, could normalization of the marker values be found. Furthermore, the survival times in the 14 patients whose postoperative CA 19-9 levels dropped to the normal range were significantly longer than in the remaining patients with persistently elevated CA 19-9 levels for the same tumor stage (Table V). This suggests that a persistently elevated postoperative serum CA 19-9 level reflects the presence of a "critical volume" of cancer. These results may confirm that such resections are only palliative and would suggest that all patients whose postoperative CA 19-9 levels remain elevated should be treated with adjuvant immunotherapy or chemotherapy, if such a practicable and efficient therapy were to be developed.

In contrast simple laparotomy and biopsy or bypass operation, without resection of the tumor, resulted in no significant change in CA 19-9 levels; after biliary decompression, however, serum CA 19-9 levels decrease for a short time, presumably as a result of improved hepatic function rather than from any regression of the cancer.[28] Following the decrease in the CA 19-9 level after resection, a secondary rise strongly suggests that a clinically significant metastatic local recurrence is involved. Of the 126 patients with resectable tumors, 116 were followed up; 113 patients developed metastatic local recurrences and 89% of them showed a secondary elevation in CA 19-9 before the lesions were visible on CT scan. The lead time of CA 19-9 ranged from 1 to 56 weeks.

Today, therefore, we believe there is evidence to support the following uses of serum CA 19-9 determinations: (1) as an adjunct in the diagnosis of patients with suspected pancreatic carcinoma, (2) as an adjunct in selecting candidates for surgical exploration to evaluate tumor resectability, (3) as an indicator for adjuvant therapy in patients with resectable tumors, (4) as a prognostic indicator before and after surgery, and (5) as a predictor of recurrences after surgical resection before these recurrences become clinically apparent.

REFERENCES

1. Moosa AR, Levin B. The diagnosis of early pancreatic cancer: The University of Chicago experience. Cancer 1981;47:1688-1697.
2. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridoma antibodies. Somat Cell Mol Genet 1979;5:957-972.

3. Magnani JL, Steplewski Z, Koprowski H, Ginsburg V. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19-9 in the sera of patients as a mucin. Cancer Res 1983;43:5489-5492.
4. Magnani JL, Nilsson B, Brockhaus M, Zopf D, Steplewski Z, Koprowski H, Ginsburg V. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is ganglioside containing sialylated lact-N-fucopentaose II. J Biol Chem 1982;257:14365-14369.
5. Safi F, Beger HG, Bittner R, Büchler M, Krautzberger W. CA 19-9 and pancreatic adenocarcinoma. Cancer 1986;57:779-783.
6. Hermanek P, Scheibe O, Spiessl B, Wagner G. TNM-Klassifikation maligner Tumoren. Berlin: Springer-Verlag, 1987.
7. Del Vilano BC, Brennan S, Brock P, Bucher C, Lui V, McClure M, Zurawaski VR. Radioimmunometric assay for a monoclonal antibody-defined tumor marker, CA 19-9. Clin Chem 1983;29:549-552.
8. Kaplan E, Meier P. Nonparametric estimation from incomparable observations. J Am Statist Assoc 1958;53:457-481.
9. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;59:163-170.
10. Armitage P. Statistical Methods in Medical Research. New York: John Wiley & Sons, 1971.
11. Lindholm L, Holmgren J, Svennerholm L, Fredman P, Nilsson O, Persson B, Myrvold M, Lagergard T. Monoclonal antibodies against gastrointestinal tumor-associated antigens isolated as monosialogangliosides. Int Arch Allergy Appl Immunol 1983;71:178-181.
12. Koprowski H, Brockhaus M, Blaszczyk M. Lewis blood-type may affect the incidence of gastrointestinal cancer. Lancet 1982;1:1332-1333.
13. Reddi K. Clinical significance of human ribonuclease. In Kawai H, ed. Clinical Features of Early Diagnosis of Pancreatic Cancer. New York: Igaku-Shion, 1980, pp 35-41.
14. Malfertheiner P, Büchler M, Kraus C, Ditschuneit H. Laktoferrin bei chronischer Pankreatitis, Pankreas Karzinom und entzündlicher Darmerkrankung. Verh Dtsch Ges Inn Med 1984;70:90-96.
15. Podolsky DK, McPhee MS, Alpert E, Warshaw AL, Isselbacher KJ. Galactosyltransferase isoenzyme II in the detection of pancreatic cancer: Comparison with radiologic, endoscopic and serologic cancer tests. N Engl J Med 1981; 304:1313-1317.
16. Mahvi D, Meyers W, Bast R. Carcinoma of the pancreas. Therapeutic efficacy as defined by a serodiagnostic test utilizing a monoclonal antibody. Ann Surg 1985;202:440-445.
17. Ohshio G, Manabe T, Watanabe Y, Endo K, Kudo H, Suzuki T, Tobe T. Comparative studies of DU-PAN-2, carcinoembryonic antigen, and CA 19-9 in the serum and bile of patients with pancreatic and biliary tract diseases: Evaluation of the influence of obstructive jaundice. Am J Gastroenterol 1990; 85:1370-1376.
18. Fabris C, Malesci A, Basso D, Bonato C, Del Favero M, Tacconi M, Meggiato T, Fogar P. Serum DU-PAN-2 in the differential diagnosis of pancreatic cancer: Influence of jaundice and liver dysfunction. Br J Cancer 1991;93:451-453.
19. Toshkov I, Mogaki M, Kazakoff K, Pour PM. The patterns of coexpression of tumor-associated antigens CA 19-9, TAG-72, and DU-PAN-2 in human pancreatic cancer. Int J Pancreatol 1994;15:97-103.
20. Tada M, Omata M, Kawai S, Saisho H, Ohto M, Randall K, Sninsky JJ. Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. Cancer Res 1993;53:2472-2474.
21. Tabata T, Fujimori T, Maeda S, Masahiro Y, Saitoh Y. The role of ras mutation in pancreatic cancer, precancerous lesions, and chronic pancreatitis. Int J Pancreatol 1993;14:237-244.
22. Masson P, Andren-Sandberg A. Crude isolation of DNA from unselected human pancreatic tissue and amplification by the polymerase chain reaction of Ki-ras oncogene to detect point mutations in pancreatic cancer. Acta Oncol 1992;31:421-424.
23. Kondo H, Sugano K, Fukayama N, Kyoguku A, Nose H, Shimada K, Ohkura H, Ohtsu A. Detection of point mutations in the K-ras oncogene at cadon 12 in pure pancreatic juice for diagnosis of pancreatic carcinoma. Cancer 1994;73:1589-1594.
24. Haglund C, Roberts PJ, Kuusela P, Scheinin TM, Mäkelä O, Jalanko H. Evaluation of CA 19-9 as a serum tumor marker in pancreatic cancer. Br J Cancer 1986;53:197-202.
25. Safi F, Roscher R, Beger HG. Tumor markers in pancreatic cancer. Sensitivity and specificity of CA 19-9. Hepatogastroenterology 1989;36:419-423.
26. Tempero MA, Uchida E, Takasaki H. Relationship of carbohydrate antigen 19-9 and Lewis antigen in pancreatic cancer. Cancer Res 1987;4:5501-5503.
27. Rosen von A, Linder S, Harmenberg U, Pegert S. Serum levels of CA 19-9 and CA 50 in relation to Lewis blood cell status in patients with malignant and benign pancreatic disease. Pancreas 1993;8:160-165.
28. Frebourg T, Bercoff E, Manchon N. The evaluation of CA 19-9 antigen level in the early detection of pancreatic cancer. A prospective study of 866 patients. Cancer 1988;62:2287-2290.

Short-Segment Barrett's Esophagus: A Prevalent Complication of Gastroesophageal Reflux Disease With Malignant Potential

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The significance of finding specialized intestinal epithelium localized to the region of the gastroesophageal junction is unclear. We tested the hypothesis that short segments of specialized intestinal epithelium are a consequence of gastroesophageal reflux disease and are premalignant. Two hundred forty-one patients with reflux symptoms underwent gastroscopy with rigorous biopsy. Barrett's esophagus was diagnosed when specialized intestinal epithelium was present on biopsy. Patients with Barrett's esophagus were subdivided according to the length of Barrett's mucosa: short-segment Barrett's (<3 cm) and extended Barrett's (≥3 cm). Esophageal function was evaluated by manometry and 24-hour pH monitoring. In another 16 patients with small noncircumferential adenocarcinomas, the endoscopic length of Barrett's mucosa was recorded. Thirty-three patients (14%) had short-segment Barrett's and 37 (15%) had extended Barrett's esophagus. Patients with short-segment Barrett's esophagus had significantly more acid exposure than patients without specialized intestinal epithelium. Eighty-one percent of patients with short-segment Barrett's esophagus had increased esophageal acid exposure as did 100% of those with extended Barrett's esophagus. All lengths of Barrett's mucosa were associated with poor esophageal sphincter function and reduced contraction amplitudes in the distal esophagus. Twelve percent of patients with short-segment Barrett's esophagus had dysplasia. The length of Barrett's mucosa was ≤3 cm in 25% (4 of 16) of patients with early Barrett's adenocarcinoma. Short-segment Barrett's esophagus is commonly associated with gastroesophageal reflux disease. Further, short segments of specialized intestinal epithelium are premalignant in nature. (J GASTROINTEST SURG 1997;1:113-122.)

It has been suggested that the normal distal esophagus may be lined with columnar mucosa for a distance of up to 2 cm,[1] placing the squamocolumnar junction within the lower esophagus. Barrett's esophagus, a premalignant condition, has traditionally been diagnosed when the columnar mucosa extends well into the esophagus, at least 3 cm proximal to the gastroesophageal junction. This emphasis on endoscopic measurements has detracted attention from the importance of the histologic morphology of the columnar lining. Specifically, identification of the presence of specialized intestinal epithelium may be critical when considering a diagnosis of Barrett's esophagus, since it is the epithelium most commonly associated with malignant change.

Barrett's esophagus is a consequence of gastroesophageal reflux disease.[2-5] Physiologic studies show that 90% of patients have a mechanically defective lower esophageal sphincter and 93% have abnormal esophageal acid exposure on pH monitoring.[3,5] It is unknown whether the presence of specialized intestinal epithelium in short segments of columnar mucosa (<3 cm) is also associated with similar pathophysiologic abnormalities.

We investigated the hypothesis that the diagnosis of Barrett's esophagus should be determined by the presence of specialized intestinal epithelium on biopsy, regardless of the length of the columnar segment present (i.e., <3 cm or ≥3 cm).

PATIENTS

Between January 1991 and February 1995, a total of 241 consecutive patients who underwent upper

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gastrointestinal endoscopy for investigation of symptoms suggestive of gastroesophageal reflux disease were studied prospectively. Patients with a history of esophageal or gastric surgery were excluded because of the possibility that the operation had distorted the anatomy of the gastroesophageal junction. Patients with a named esophageal motor abnormality (i.e., achalasia or scleroderma) were also excluded. The patients had multiple biopsies obtained from the lower esophagus according to the protocol outlined in the Methods section. Only patients whose biopsies showed columnar mucosa containing specialized intestinal epithelium were classified as having Barrett's esophagus. The patients were grouped on the basis of their biopsy findings as follows: (1) patients with short-segment Barrett's esophagus—that is, columnar lining <3 cm in length with specialized intestinal epithelium; (2) patients with extended Barrett's esophagus—that is, a columnar lining ≥3 cm in length with specialized intestinal epithelium; and (3) patients without Barrett's esophagus—that is, patients who did not have a columnar-lined esophagus or those with a columnar lining but without specialized intestinal epithelium regardless of the length of the esophagus.

Of the 241 patients in the study population who underwent gastroscopy, 211 volunteered for esophageal manometry and 204 had 24-hour esophageal pH monitoring. The findings on stationary manometry and 24-hour pH monitoring were compared among the three groups and with similar observations made in 50 healthy volunteers who served as control subjects.

A second study of the length of Barrett's esophagus was undertaken in 16 patients with small Barrett's adenocarcinomas who underwent endoscopic examination. The patients were drawn from a larger population of patients presenting with adenocarcinoma. The criterion for selection was a noncircumferential tumor measuring ≤3 cm in length. It was thought that endoscopic measurement of the length of Barrett's mucosa would be most accurate in such patients.

METHODS

Stationary Manometry

Stationary manometry was performed after an overnight fast using a single catheter assembly consisting of five polyethylene tubes bonded together with five lateral openings placed at 5 cm intervals from the distal end and oriented radially around the circumference. The catheter was perfused with distilled water at a constant rate of 0.6 ml/min using a pneumohydraulic low-compliance perfusion pump (Arndorfer Medical Specialties, Greendale, Wis.). The catheter was withdrawn at 1 cm intervals every 20 seconds and the polygraph recordings from the five channels were analyzed by hand. Lower esophageal sphincter pressure was measured at the respiratory inversion point in a manner previously described[6] and resting sphincter pressure, overall length, and abdominal length were calculated from the mean of the five recordings. A structurally defective sphincter was defined as any one or more of the following: resting pressure <6 mm Hg, overall length <2 cm, or abdominal length <1 cm.[6]

Esophageal body function was assessed with the catheter positioned with the proximal opening 1 cm below the lower border of the upper esophageal sphincter and the other four openings trailing over the length of the esophageal body. The patients performed 10 wet swallows with 5 ml liquid boluses. The median amplitudes of contraction were determined at each of five levels in the esophageal body from the 10 wet swallows. The presence of simultaneous waves was diagnosed when the rate of wave progression (from peak to peak) was greater than 20 cm/sec.

24-Hour Esophageal pH Monitoring

Esophageal pH monitoring was performed using a glass electrode (Ingold, Switzerland) placed 5 cm above the upper border of the manometrically defined lower esophageal sphincter. The subjects were instructed to carry out their normal daily activities but to avoid strenuous exertion. During the day they were asked to remain in the upright position and were given a diet sheet indicating foods with a pH in the range of 5 to 7. The subjects were asked to note the times when they ate their meals, when they went to sleep, and when they arose the following morning. All medications known to interfere with gastrointestinal motility or secretion were discontinued for 48 hours prior to the study (omeprazole was stopped for 2 weeks), and smoking and alcohol were prohibited during the recording period. The pH recordings were stored on a portable Digitrapper (Synectics Medical, Inc., Irving, Tex.) and downloaded to an IBM personal computer for analysis. Esophageal acid exposure was analyzed by a computer program (Gastrosoft, Dallas, Tex.) using both a scoring system previously described by Johnson and DeMeester[7] and by recording the total percentage of time that was spent at pH <4. The composite score for pH <4 of 14.9 lies at the ninety-fifth percentile in normal subjects, and levels higher than this are indicative of abnormal esophageal acid exposure. The normal total percentage of time spent at pH <4 is less than 4.4%.

Endoscopy

Upper gastrointestinal endoscopy was performed in all patients by the senior author (T.R.D). The po-

sition of the crural impression (emphasized by asking the patient to sniff), the position of the anatomic gastroesophageal junction (identified as the level where the more dilated stomach with vertically running rugal folds became the tubular esophagus with smooth mucosa), and the most proximal limit of the squamocolumnar junction were recorded. The presence of hiatal hernia was defined endoscopically when the difference between the position of the crura and the gastroesophageal junction was 3 cm or more.

Biopsies were obtained from the white squamous epithelium of the distal esophagus in all subjects. Biopsies were also obtained from columnar epithelium in one of two ways: (1) In patients with macroscopic evidence of columnar mucosa in the esophagus, biopsies were obtained from the four quadrants of the esophagus every 2 cm along the visual length of the columnar mucosa, and (2) in patients with no obvious columnar lining in the lower esophagus, three biopsies were taken during a retroflex view from a location 1 cm below the squamocolumnar junction. The location of each biopsy was recorded as the distance from the incisors. The presence of specialized intestinal epithelium on biopsy confirmed the diagnosis of Barrett's esophagus. The length of the Barrett's mucosa was the difference between the location of the gastroesophageal junction and the location of the highest biopsy containing specialized intestinal epithelium. Columnar mucosa that extended more than 3 cm above the gastroesophageal junction but did not contain specialized intestinal epithelium on biopsy was not considered to be Barrett's mucosa for the purpose of this study.

Esophagitis was graded according to Savary-Miller criteria. Grade I was not considered to represent esophagitis. An esophageal stricture was identified by the inability to pass a 12 mm diameter endoscope with ease.

Complications

Complications arising in patients with Barrett's esophagus included the presence of stricture or deep ulceration on endoscopy and the identification of dysplasia histologically. Dysplasia was defined according to the criteria for inflammatory bowel disease.[8] Indefinite dysplasia was not considered a complication.

Statistics

Comparisons of proportions were performed using Fisher's exact test or chi-square analysis. Differences in nonparametric data between multiple groups were identified by the Kruskal-Wallis test and comparisons between individual groups were made using the Mann-Whitney U test. Values are expressed as medians. Significance was taken at the 5% level.

RESULTS

Demographic data of each of the two study groups and the normal controls are shown in Table I. From the first study 33 patients (14%) had short-segment Barrett's disease (i.e., columnar lining <3 cm in length with specialized intestinal epithelium), 37 patients (15%) had extended Barrett's disease (i.e., a columnar lining ≥3 cm in length with specialized intestinal epithelium), and 171 patients (71%) had no specialized intestinal epithelium.

Lower Esophageal Sphincter Characteristics

Table II shows the individual lower esophageal sphincter components for the patients in the first study. Fig. 1 illustrates the prevalence of a mechanically defective lower esophageal sphincter (LES)—that is, the percentage of patients having one or more

Table I. Demographic data

	No.	M/F	Age (yr) Median	Age (yr) Range
Normal volunteers	50	20/30	31*	23-71
No Barrett's	171	96/75	48	15-89
Short-segment Barrett's	33	25/8†,‡	53	20-79
Extended Barrett's	37	29/8†,§	51	35-80
Barrett's adenocarcinoma	16	15/1†,§	62.5‖	42-80

*Significant difference in ages between the groups ($\chi 2 = 55.7$, 4 df, $P < 0.01$, Kruskal-Wallis). All groups significantly older than volunteers ($P < 0.05$, Mann-Whitney U test).
†$P < 0.01$ vs. normals (Fisher's exact test).
‡$P = 0.05$ vs. No Barrett's.
§$P < 0.05$ vs. No Barrett's.
‖Significantly older than the other three patient groups ($P < 0.05$, Mann-Whitney U test).

Table II. Prevalence of abnormal lower esophageal sphincter components

Component	Volunteers (n = 50)	No Barrett's (n = 146)	Barrett's <3 cm (n = 32)	Barrett's ≥3 cm (n = 33)
Resting pressure <6 mm Hg	0 (0%)*	36 (25%)	17 (43%)†	24 (73%)†
Overall length <2 cm	0 (0%)*	26 (18%)	9 (28%)	13 (39%)‡
Abdominal length <1 cm	1 (2%)*	40 (27%)	10 (27%)	20 (61%)§

*P <0.05 vs. each of the patient groups (Fisher's exact test).
†P <0.01 vs. No Barrett's group (χ^2).
‡P <0.05 vs. No Barrett's group (χ^2).
§P <0.01 vs. No Barrett's group and Barrett's <3 cm (χ^2).

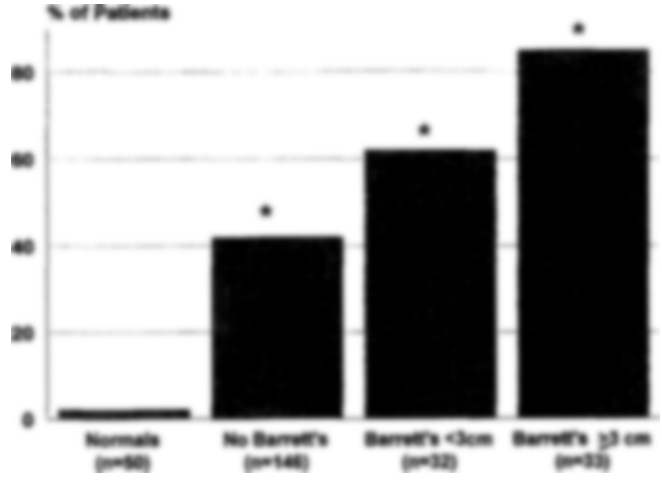

Fig. 1. Prevalence of a mechanically defective lower esophageal sphincter. * = all patient groups were significantly different from normals (P <0.01) and from each other ($P \leq 0.05$).

abnormal sphincter components. Only one of the healthy volunteers had a mechanically defective LES. There was a progressive increase in the prevalence of a mechanically defective LES from the patients without specialized intestinal epithelium to the patients with short-segment Barrett's esophagus to patients with extended Barrett's esophagus (Fig. 1).

Fig. 2 illustrates the scatter plot of the LES resting pressures. Each of the patient groups had reduced mean lower esophageal sphincter pressures compared to control subjects. The patients with short-segment Barrett's esophagus had lower LES pressure compared to the patients without specialized intestinal epithelium, whereas the patients with extended Barrett's esophagus had lower LES pressures than those with short-segment Barrett's disease.

Fig. 3 shows the overall length and abdominal length of the LES in each of the patients in study 1. No difference was observed between the patients without specialized intestinal epithelium and those with short-segment Barrett's disease. The patients with extended Barrett's disease had shorter overall sphincter lengths than the patients without specialized intestinal epithelium and shorter abdominal sphincter lengths than both the patients without specialized intestinal epithelium and those with short-segment Barrett's esophagus.

Esophageal Acid Exposure

Increased esophageal acid exposure (composite score >14.9) was present in 59% of patients without specialized intestinal epithelium, 81% of patients with short-segment Barrett's esophagus, and 100% of patients with extended Barrett's esophagus (Fig. 4). Fig. 5 shows a scatter plot of the percentage of time spent at

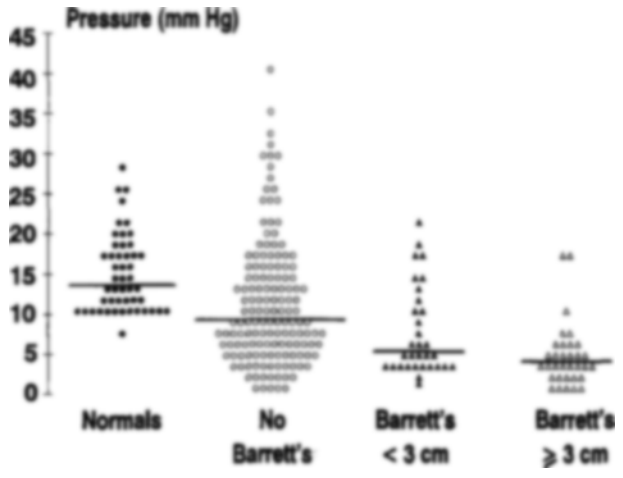

Fig. 2. Scatter plot of the lower esophageal sphincter pressure in each of the study groups. Horizontal line indicates the median. There was a significant difference between the groups (χ^2 = 61.7, 3 df, P <0.01, Kruskal-Wallis). All groups were significantly different (P <0.05, Mann-Whitney).

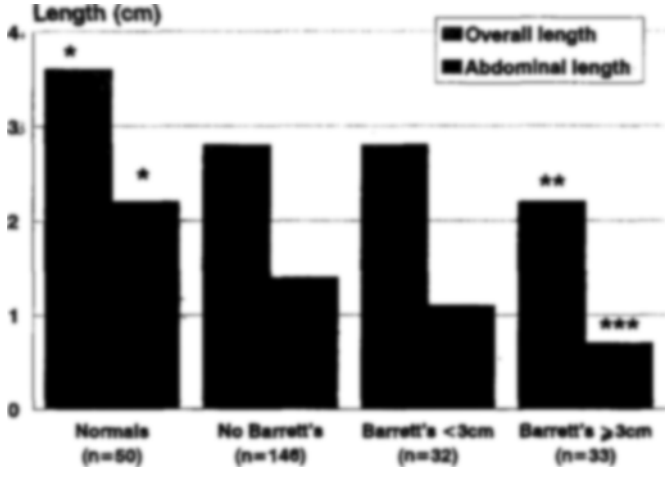

Fig. 3. Overall length and abdominal length of the lower esophageal sphincter. Bar represents the median. * = all groups were significantly different from normals (P <0.01 for both parameters). For overall length No Barrett's vs. extended Barrett's, P <0.05. For abdominal length extended Barrett's vs. No Barrett's, P <0.01, and extended Barrett's vs. short Barrett's, P <0.05.

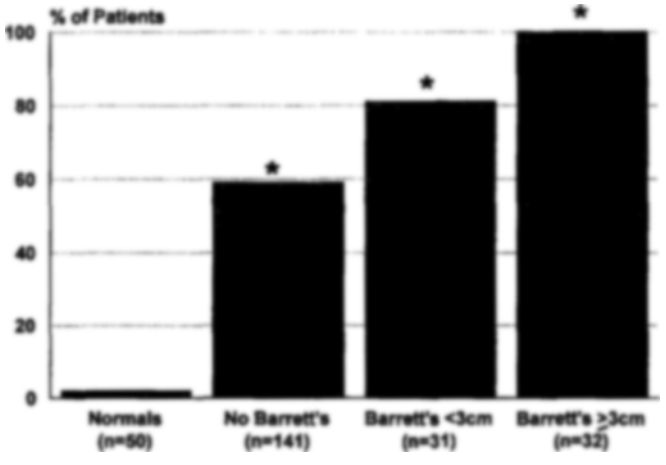

Fig. 4. Prevalence of a positive composite score for increased esophageal acid exposure. * = all groups were significantly different from normals (P <0.01) and from each other ($P \leq 0.05$).

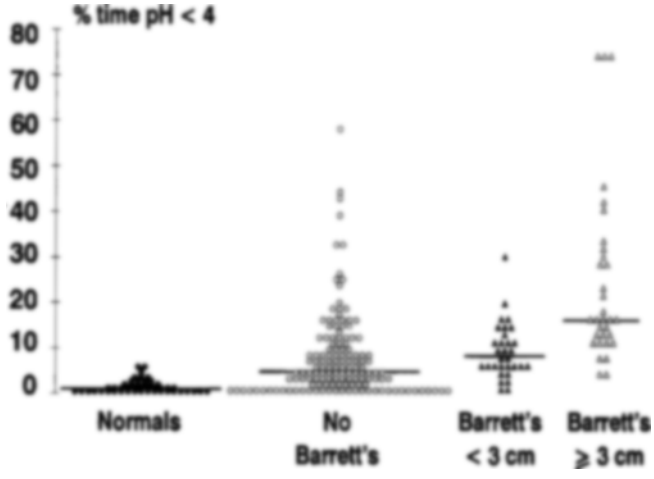

Fig. 5. Scatter plot of the total percentage of time the esophageal pH was <4 in each of the study groups. Horizontal line indicates the median. There was a significant difference between the groups ($\chi^2 = 82.6$, 3 df, $P < 0.01$, Kruskal-Wallis). All groups were significantly different from each other ($P < 0.01$, Mann-Whitney).

pH <4 for the patients in study 1. Patients with short-segment Barrett's esophagus had significantly greater esophageal acid exposure than those without specialized intestinal epithelium. However, the percentage of time that the esophageal pH was <4 was significantly higher in patients with extended Barrett's compared to patients with short-segment Barrett's esophagus.

Esophageal Body Function

Amplitude of esophageal body contraction in the distal esophagus was significantly reduced in all of the patient groups compared to the normal volunteers at all five levels in the esophagus (Fig. 6). In patients with short-segment and extended Barrett's esophagus, there was a significant reduction in contraction amplitudes in the lower esophagus compared to patients without specialized intestinal epithelium. The prevalence of an increased number of simultaneous waves (20% or more between two channels) was 2% in normal subjects, 23% in patients without specialized intestinal epithelium, 31% in patients with short-segment Barrett's, and 15% in patients with extended Barrett's esophagus. All patient groups had a significantly greater prevalence compared to normal controls ($P < 0.01$, chi-square analysis).

Hiatal Hernia

The prevalence of endoscopically diagnosed hiatal hernia was 34% (57 of 171) in patients without specialized intestinal epithelium, 55% (18 of 33) in patients with short-segment Barrett's, and 78% (29 of 37) in patients with extended Barrett's esophagus. Groups differed significantly from one another ($P < 0.05$).

Complications

The prevalence of complications is shown in Table III. The prevalence of dysplasia did not differ between patients with short-segment and extended Barrett's esophagus. Four patients (12%) with short-segment Barrett's esophagus had dysplasia, which was high grade in two and low grade in two, whereas dysplasia was observed in 7 (19%) of 37 patients with extended Barrett's esophagus, high grade in one and low grade in six. Dysplasia was not seen in patients with a columnar-lined esophagus in the absence of specialized intestinal epithelium.

Malignancy

In the second study, in 25% (4 of 16) of patients with small noncircumferential Barrett's adenocarcinomas measuring 3 cm or less in length, the lesions were associated with short-segment Barrett's metaplasia (Table IV). In all four patients in whom the adenocarcinoma was associated with a short-segment of Barrett's mucosa, there was histologic evidence of high-grade dysplasia within areas of the specialized intestinal epithelium, suggesting that the metaplastic mucosa was the source of the adenocarcinoma.

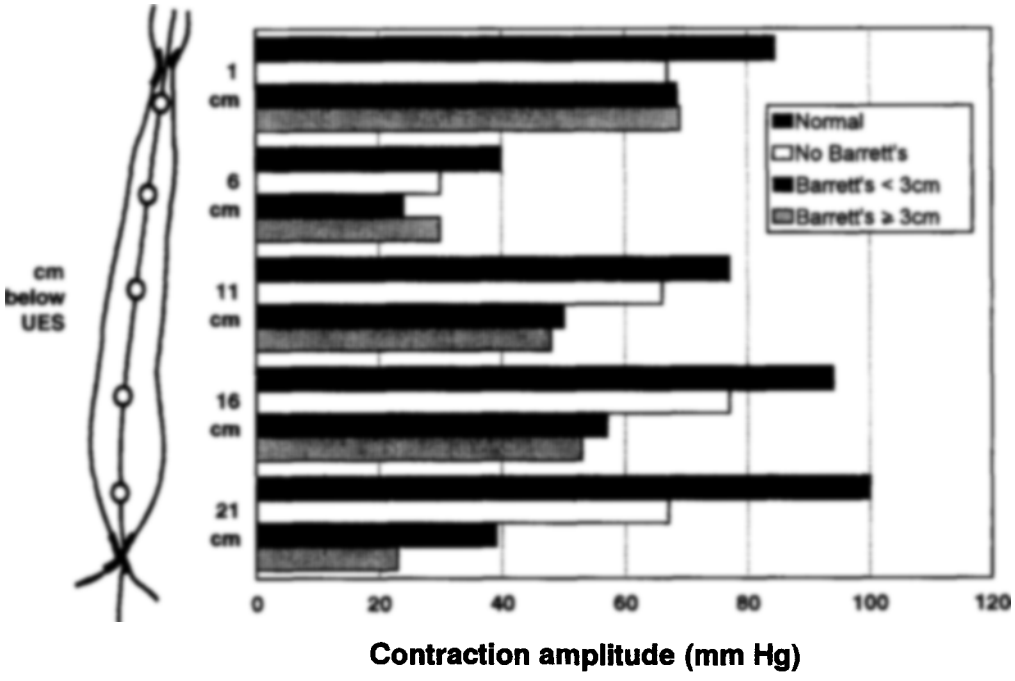

Fig. 6. Median contraction amplitudes at each of the five levels of the esophagus for the different groups (cm below the lower border of the upper esophageal sphincter [UES]). There were significant differences between the groups at each of the five levels (Kruskal-Wallis, 3 df, 1 cm $\chi^2 = 10$, 6 cm $\chi^2 = 17$, 11 cm $\chi^2 = 14.4$, 16 cm $\chi^2 = 24.7$, and 21 cm $\chi^2 = 26.1$; $P < 0.01$). All patient observations were significantly different from normals (Mann-Whitney, $P < 0.05$) at the lower four levels. Additionally, the short-segment Barrett's group was significantly different from the No Barrett's group at the 6, 16, and 21 cm levels ($P < 0.05$, Mann-Whitney). The extended Barrett's group was significantly different from the No Barrett's group at the lower three levels ($P < 0.01$ Mann-Whitney).

Table III. Esophageal complications*

Group	Esophagitis†	Stricture	Ulcer	Dysplasia
No Barrett's (n = 171)	42 (25%)	21 (12%)	4 (2%)	0
Barrett's <3 cm (n = 33)	13 (39%)	4 (12%)	0	4 (12%) (2 high grade, 2 low grade)
Barrett's ≥3 cm (n = 37)	20 (54%)‡	7 (19%)	3 (8%)	7 (19%) (1 high grade, 6 low grade)

*Individual patients may have had more than one of the complications.
†Savary-Miller grade II or III.
‡$P < 0.01$ vs. No Barrett's group.

Table IV. Endoscopic and histological characteristics in patients with small Barrett's adenocarcinomas

Patient	Endoscopic length of tumor (cm)*	Endoscopic length of Barrett's (cm)†	Appearance of Barrett's	Histologic evidence of dysplasia
1	2	Microscopic		High grade
2	3	Microscopic		High grade
3	1	1	Circumferential	High grade
4	3	2	Tongue	High grade
5	None	5	Circumferential	High grade
6	3	5	Circumferential	High grade
7	1	6	Circumferential	No
8	1	6	Circumferential	High grade
9	1	7	Circumferential	High grade
10	None	7	Circumferential	High grade
11	3	8	Circumferential	High grade
12	None	9	Circumferential	High grade
13	None	10	Circumferential	High grade
14	2	10	Circumferential	High grade
15	3	11	Circumferential	Low grade
16	2	12	Circumferential	No

*None refers to invasive intramucosal carcinomas detected histologically in the absence of any obvious mucosal abnormality.
†In two tumors no Barrett's mucosa was seen endoscopically, but specialized intestinal epithelium was present at the margins of the tumor on histologic examination.

DISCUSSION

It has become recognized that the region of the gastroesophageal junction may harbor areas of specialized intestinal epithelium.[9] In this situation the metaplastic mucosa is localized to within 2 cm of the gastroesophageal junction and may not be readily apparent to the naked eye. Spechler et al.[10,11] indicated that such short lengths of specialized intestinal epithelium are found on distal esophageal biopsies in up to 18% of all patients undergoing upper gastrointestinal endoscopy. The authors indicated that this phenomenon is common and unrelated to gastroesophageal reflux disease. This implication must be guarded in that a detailed physiologic assessment of the patients was not undertaken.

In our study, esophageal function testing indicated that most patients (81%) with short segments of specialized intestinal epithelium had a high prevalence of gastroesophageal reflux disease, which was proved by means of 24-hour pH monitoring. Furthermore, these patients had the potential for complications and the development of adenocarcinoma.

The reason for this increased esophageal acid exposure is a mechanically defective sphincter. That is, patients with short-segment and extended Barrett's esophagus had a higher prevalence of a defective sphincter compared to control subjects and patients with no specialized intestinal epithelium. The resting LES pressures showed a progressive decrease from those patients with no specialized intestinal epithelium to those patients with short-segment Barrett's to those with extended Barrett's esophagus. However, patients with short-segment Barrett's compared to those with extended Barrett's esophagus had longer sphincters and tended to have an adequate length of sphincter exposed to the intra-abdominal pressure environment. The preservation of these components may reflect that these patients were still in the early stages of disease and were maintaining some LES function resulting in less esophageal acid exposure. Longitudinal studies are needed to confirm this hypothesis.

Analysis of esophageal body motility indicated that the groups with short-segment and extended Barrett's esophagus had a reduction in the amplitude of the contractions and a higher incidence of simultaneous waveforms in the esophageal body. These observations indicate that patients with Barrett's esophagus have a significant motility disorder of the esophageal body. The latter suggests that the alteration in esophageal body motility is primary rather than secondary to the loss of LES function. This observation along with recent findings of increased esophageal exposure to duodenal juice suggests that patients with Barrett's esophagus are unique in that they have a more global foregut motility abnormality.

Both patients with short-segment and those with

extended Barrett's esophagus were subject to the development of complications. The risk of benign complications was highest in the groups with extended Barrett's esophagus; however, dysplasia was identified in all lengths of Barrett's mucosa. Two patients with short-segment Barrett's esophagus underwent esophagectomy for high-grade dysplasia. These observations are at odds with the findings of Iftikhar et al.,[12] who largely discounted the risk of dysplastic change in short lengths of Barrett's mucosa. However, they required that patients have >5 cm of circumferential Barrett's mucosa to enter their study and did not focus on patients with shorter lengths of Barrett's mucosa.

Esophageal adenocarcinoma is the fastest rising cancer in the United States, and the incidence of adenocarcinomas of the "gastric cardia" is increasing in a parallel fashion.[13-15] The epidemiology of these cancers is similar; both affect elderly white males who often have a history of gastroesophageal reflux disease.[16-19] Adenocarcinomas of the esophagus are associated with specialized intestinal epithelium in more than 75% of cases and adenocarcinomas of the cardia are associated with specialized intestinal epithelium in 42% to 73% of patients.[19,20] Furthermore, Schnell et al.[21] found four cases of early distal esophageal adenocarcinomas arising within short segments of intestinal epithelium. On the basis of these two observations it is postulated that adenocarcinoma of the gastric cardia arises from short segments of specialized intestinal epithelium located at the gastroesophageal junction.

In the present study a series of patients with small noncircumferential Barrett's adenocarcinomas (≤3 cm long; many of whom had intramucosal carcinoma) were analyzed to assess the macroscopic extent of the length of the Barrett's mucosa. Twenty-five percent had adenocarcinomas arising in Barrett's mucosa, which was 3 cm in length. High-grade dysplasia was present within the specialized intestinal epithelium in each of these tumors suggesting a Barrett's etiology. Many tumors at the gastroesophageal junction may originate from short segments of Barrett's mucosa; however, they may cannibalize the specialized intestinal epithelium making it impossible to determine the source of the tumor from the resected specimen. If this is true, the association between chronic gastroesophageal reflux disease and adenocarcinoma may be higher than is currently documented.

This study indicates that when specialized intestinal epithelium is present, even when specialized intestinal epithelium is restricted to the region of the gastroesophageal junction, the endoscopist should confidently make the diagnosis of a premalignant condition. We conclude that the 3 cm rule represents a restrictive definition of Barrett's esophagus and is no longer appropriate in the presence of specialized intestinal columnar epithelium. Patients with specialized intestinal epithelium at the gastroesophageal junction are at risk of developing adenocarcinoma of the cardia. Further studies are necessary to establish the precise magnitude of this risk and the need for endoscopic surveillance of this group of patients.

REFERENCES

1. Hayward J. The lower end of the esophagus. Thorax 1961; 16:36-41.
2. DeMeester TR, Attwood SEA, Smyrk TC, et al. Surgical therapy in Barrett's esophagus. Ann Surg 1990;212:528-542.
3. Iscone C, DeMeester TR, Little AG, et al. Barrett's esophagus. Functional assessment, proposed pathogenesis and surgical therapy. Arch Surg 1983;118:543-549.
4. Winters C Jr, Spurling TJ, Chobanian SJ, et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-124.
5. Attwood SEA, DeMeester TR, Bremner CG, et al. Alkaline gastroesophageal reflux: Implications in the development of complications in Barrett's columnar-lined lower esophagus. Surgery 1989;106:764-770.
6. Zaninotto G, DeMeester TR, Schwizer W. The lower esophageal sphincter in health and disease. Am J Surg 1988;155:104-110.
7. Johnson LF, DeMeester TR. Development of the 24 hour intraesophageal pH monitoring composite scoring system. J Clin Gastroenterol 1986;8:52-58.
8. Riddell RH, Goldman H, Ranschoff DF, et al. Dysplasia in inflammatory bowel disease: Standardized classification with provisional clinical applications. Hum Pathol 1983;14:931-968.
9. Clark GWB, Ireland AP, Chandrasoma P, et al. Inflammation and metaplasia in the transitional epithelium of the gastroesophageal junction: A new marker for gastroesophageal reflux disease [abst]. Gastroenterology 1994;106:A63.
10. Spechler SJ, Zeroogian JM, Antonioli DA, et al. Prevalence of metaplasia at the gastroesophageal junction. Lancet 1994; 344:1533-1536.
11. Spechler SJ, Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. Gastroenterology 1996;110:614-621.
12. Iftikhar SY, James PD, Steele RJC, et al. Length of Barrett's esophagus: An important factor in the development of dysplasia and adenocarcinoma. Gut 1992;33:1155-1158.
13. Hesketh PJ, Clapp RW, Doos WG, et al. The increasing frequency of adenocarcinoma of the esophagus. Cancer 1989; 64:526-530.
14. Yang PC, Davis S. Incidence of cancer of the esophagus in the U.S. by histological type. Cancer 1988;61:612-617.
15. Blot WJ, Devesa SS, Kneller RW, et al. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287-1289.
16. Wang HH, Antonioli DA, Goldman H. Comparative features of esophageal and gastric adenocarcinomas: Recent changes in type and frequency. Hum Pathol 1986;17:482-487.
17. Kalish RJ, Clancy PE, Orringer MB, et al. Clinical, epidemiologic and morphologic comparison between adenocarcino-

mas arising in Barrett's esophageal mucosa and in the gastric cardia. Gastroenterology 1984;86:461-467.
18. Haggit RC, Tryzelaar J, Ellis FH, et al. Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. Am J Clin Pathol 1979;70:1-5.
19. Cameron AJ, Lomboy CT, Pera M, et al. Adenocarcinoma of the esophagogastric junction and Barrett's esophagus. Gastroenterology 1995;109:1541-1546.
20. Clark GWB, Smyrk TC, Burdiles P, et al. Is Barrett's metaplasia the source of adenocarcinomas of the cardia? Arch Surg 1994;129:609-614.
21. Schnell TG, Sontag SJ, Chejfec G. Adenocarcinomas arising in tongues or short segments of Barrett's esophagus. Dig Dis Sci 1992;37:137-143.

Portal Vein Thrombosis in Cirrhosis With Variceal Hemorrhage

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Organized thrombus in the main trunk of the portal vein was encountered in 85 (6.5%) of 1300 patients with cirrhosis and variceal hemorrhage who underwent direct portacaval shunt (PCS). The thrombus was successfully removed with restoration of portal blood flow in all patients by phlebothrombectomy and balloon catheter extraction. Of the 85 patients, 65 were among 400 unselected patients who underwent emergency PCS (16%), and 20 were among 900 selected patients who underwent elective PCS (2%). All patients were closely followed for at least 5 years. Patients with portal vein thrombosis (PVT) had more advanced liver disease than those without PVT, reflected preoperatively in significantly higher ($P < 0.01$) incidences of ascites (75%), severe muscle wasting (52%), varices of very large size (94%), the hyperdynamic state (94%), severe hypersplenism with a platelet count of less than 50,000/mm^3 (92%), and placement in Child's class C (52%). Side-to-side PCS reduced the portal vein–inferior vena cava pressure gradient to a mean of 23 mm saline solution in patients with PVT, similar to the marked pressure reduction obtained in patients without PVT. PCS promptly stopped variceal bleeding in all patients in the emergency PCS group. Permanent prevention of recurrent variceal bleeding was successful in 95% of patients with PVT and more than 99% of patients without PVT. Survival rates were similar in patients with and without PVT. In patients with PVT, survival rates at 30 days and 1, 5, 10, and 15 years following emergency PCS were 69%, 66%, 65%, 55%, and 51%, respectively, and following elective PCS were 95%, 90%, 70%, 65%, and 60%, respectively. Quality of life was similar in patients with and without PVT. Long-term PCS patency was demonstrated yearly in 93% of patients in the group with PVT and in 99.7% of patients without PVT. Other similarities after 5 years between patients with and without PVT, respectively, were the incidences of recurrent encephalopathy (9% vs. 8%), alcohol abstinence (61% vs. 64%), improved liver function (68% vs. 62% to 75%), and return to work (52% vs. 56% to 64%). It was concluded that in patients with cirrhosis and variceal hemorrhage it is almost always possible to remove portal vein thrombus by means of phlebothrombectomy and then perform a direct PCS with results similar to those achieved in the absence of PVT. (J GASTROINTEST SURG 1997;1:123-131.)

Thrombosis of the main trunk of the portal vein in cirrhosis is not uncommon. It is often discovered in association with an episode of variceal hemorrhage. Presumably the thrombosis results from stagnation of blood throughout the portal system caused by intrahepatic obstruction to portal blood flow. However, little has been written about therapy for variceal bleeding in patients with cirrhosis and portal vein thrombosis, and no well-defined treatment strategy has been developed or considered.

From 1963 to 1990 we performed portacaval shunt (PCS) as an emergency procedure for treatment of acutely bleeding esophagogastric varices in 400 unselected patients with cirrhosis.[1] From 1958 to 1991 we performed PCS electively in 900 selected patients who were referred to us after they recovered from an episode of bleeding esophagogastric varices. These 1300 patients were studied prospectively and all of them underwent at least 5 years of close follow-up. At the time of the PCS operation, well-organized thrombus was discovered in the main trunk of the portal vein in 85 patients (6.5%). This is a report of the course of these 85 patients with portal vein thrombosis (PVT), and it includes data on early and long-term

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survival, control of bleeding, incidence of portal systemic encephalopathy (PSE), and quality of life.

PATIENTS AND METHODS

Study Groups

The 400 acutely bleeding patients who underwent emergency PCS have been described in detail in a recent report.[1] The distinguishing characteristics of the emergency PCS study group included the following: (1) they were unselected so that "all comers" with acute variceal hemorrhage were included; (2) a direct PCS between the portal vein (PV) and the inferior vena cava (IVC) was undertaken within 8 hours of initial contact; and (3) they were studied prospectively with data being collected on line. Details of the study and treatment plans have been reported previously.[2-7] Sixty-five (16%) of these 400 acutely bleeding patients were found at operation to have well-organized thrombus in the PV.

The 900 patients who underwent elective PCS were referred for surgery after recovering from an episode of variceal hemorrhage, usually at a hospital other than our own. As is true of all studies of elective PCS, the patients were highly selected. Twenty (2%) of these 900 patients were found at operation to have well-organized thrombus in the PV.

Diagnostic Workup

The diagnostic workup was completed within 2 to 7 hours of initial contact in the acutely bleeding patients who underwent emergency PCS. Other than the rapidity of performance, the diagnostic workup in the elective PCS group was similar to that in the emergency PCS group. The diagnostic workup has been described previously.[1-7] The cornerstone of the diagnostic workup was esophagogastroduodenoscopy. Arteriography with indirect portography was performed routinely from 1969 to 1976, at which time it was discontinued as a routine procedure. PVT was not demonstrated or suspected during the diagnostic workup. As soon as the results of the diagnostic studies were known, patients were assigned to Child's risk classes based on the five criteria originally proposed by Child and Turcotte[8] for assessing hepatic functional reserve. The widely used quantitative method of Campbell et al.[9] was used to reduce the subjectivity of Child's criteria by assigning severity points to each criterion and calculating a total point score.

Phlebothrombectomy

A long segment of PV was dissected free from the level of the bifurcation in the liver hilum to a point where it disappeared behind the pancreas. A small wedge of pancreas was sometimes excised to attain maximal length of the vein. The PV was occluded with vascular clamps at the level of the bifurcation above and the pancreas below. A longitudinal incision, 2.0 to 2.5 cm in length, was made in the posterolateral wall of the PV at the site of the future side-to-side PCS. The organized thrombus was then extracted using endarterectomy instruments, particularly the spatula, and gentle traction on the thrombus. Finally, a Fogarty balloon catheter was inserted above and then below to extract any remaining thrombus and debris. Phlebothrombectomy was not terminated until brisk blood flow was obtained from both ends of the venotomy. Heparin solution was instilled in the PV beyond the occluding vascular clamps.

Portacaval Shunt Operation

Our technique of direct PCS has been described in detail elsewhere.[10] Intraoperative pressure measurements were obtained before and after the shunt procedure by direct needle puncture of the PV and IVC with the use of a saline solution manometer positioned at the level of the IVC. The postshunt PV-IVC pressure gradient was less than 50 mm of saline solution in 97% of patients. Cardiac output was measured before and after construction of the venous anastomosis. A large wedge liver biopsy was obtained from all patients and confirmed the diagnosis of cirrhosis in each case. Side-to-side PCS was performed in 1174 patients, end-to-side PCS was performed in 122, and another type of shunt was used in four patients after attempts at conventional portacaval anastomosis were unsuccessful.

Postoperative Therapy

The patients were admitted to a surgical intensive care unit where monitoring consisted of serial measurements of cardiovascular, pulmonary, hepatic, and renal function; fluid, electrolyte, and acid-base balance; blood count; and blood coagulation. Daily neomycin therapy, cathartics, and enemas were continued for 3 days in patients in the emergency PCS group. Hypokalemic alkalosis was treated vigorously by intravenous administration of potassium chloride. All patients received intensive respiratory therapy. An H_2-receptor antagonist, when these became available, and antacids were given throughout the postoperative course to counteract and suppress gastric acid secretion and prevent stress ulceration of the stomach. Delirium tremens was treated with parenteral administration of magnesium sulfate, diazepam, or chlordiazepoxide hydrochloride, and 50% dextrose solution with vitamins. Oral nutrition progressed until a

diet that contained 80 g protein per day was tolerated. Dietary protein tolerance was carefully evaluated. The patients and their families were given detailed dietary instructions by a dietitian and they also received repeated counseling concerning abstinence from alcohol. The patients were discharged from the hospital on a 3000-calorie diet limited to 60 g protein and 2 g sodium salt per day.

Lifelong Follow-Up

The patients were seen in the portal hypertension clinic monthly for the first postoperative year and every 3 months thereafter. At each clinic visit, clinical status was evaluated and the presence or absence of PSE was determined by a battery of tests that included a series of timed number connection tests, evaluation of mental status by a physician, examination for asterixis, and measurement of arterial blood ammonia. In addition, measurements were made of blood count, hepatic function, renal function, and fluid and electrolyte balance. A dietitian interviewed the patients and their families at each clinic visit and counseled them on restricting protein intake to 60 g/day and sodium intake to 2 g/day. Each year PCS patency and function were assessed by Doppler duplex ultrasonography or shunt catheterization with pressure measurements and angiography. Needle biopsy of the liver was performed intermittently during the follow-up period. The 1-, 5-, and 10-year follow-up rates were 10%, 96%, and 94%, respectively. All of the patients had been operated on 5 or more years earlier.

Data Collection and Analysis

Beginning with initial contact and continuing through lifelong follow-up evaluation, 220 categories of data were recorded on standard forms and entered into a computer program for analysis. Statistical significance was determined by Student's *t* test, analysis of variance for numeric variables, and chi-square test with Yates' correction for nonnumeric variables. Kaplan-Meier survival estimates and log-rank survival comparisons were computed using SAS version 6.09 software. Kaplan-Meier survival data are presented in the graph. In addition, survival data adjusted by age and sex for the California population are presented in Tables I to V.

RESULTS

Clinical Characteristics

The clinical characteristics of the 85 patients with PVT are compared with those of the 1215 patients without PVT in Table I. Patients with PVT ranged in age from 33 to 75 years, which is similar to the age range in patients without PVT in the emergency PCS group (22 to 75 years) and in the elective PCS group (26 to 80 years). Patients with and without PVT were not significantly different with regard to sex, number of variceal bleeding episodes, alcoholism, incidence of jaundice, and incidence of delirium tremens on initial contact or documented history. Forty percent of patients with PVT were found to have PSE on initial contact or had a documented history, which was not significantly different from the 33% incidence of PSE in patients without PVT. Nevertheless, patients with

Table I. Clinical characteristics of patients with and without portal vein thrombosis

Characteristics	With PVT (n = 85) (%)	Without PVT: Emergency PCS (n = 335) (%)	Without PVT: Elective PCS (n = 880) (%)
Male sex	71	69	68
Two or more bleeding episodes	62	44	55
Ascites	75*	53	63
Jaundice	60	50	55
Severe muscle wasting	52*	37	39
PSE on initial contact and/or documented history	40	33	33
Delirium tremens on initial contact and/or documented history	28	23	16
Child's risk class			
A	0	11	11
B	48	65	58
C	52*	24	31

*$P < 0.01$.

Table II. Results of preoperative diagnostic studies in patients with and without portal vein thrombosis

Laboratory results	With PVT (n = 85) (%)	Without PVT: Emergency PCS (n = 335) (%)	Without PVT: Elective PCS (n = 880) (%)
Varices on endoscopy and/or x-ray film	100	100	100
Very large varices (3 + on 0 to 3 + scale)	94*	52	40
Cirrhosis proved by biopsy	100	100	100
Alcoholic	82	95	86
Other	18	5	14
Hyperdynamic state (cardiac output ≥6 L/min)	94*	89	74
Hypersplenism (platelet count <50,000/mm^3)	92*	61	41

*P <0.01.

Table III. Operative data in patients with and without portal vein thrombosis

Operative data	With PVT (n = 85) (%)	Without PVT: Emergency PCS (n = 335) (%)	Without PVT: Elective PCS (n = 880) (%)
Mean PV-IVC gradient ± SEM			
Pre-PCS (mm saline)	285 ± 7	271 ± 7	254 ± 8
Post-PCS (mm saline)	23 ± 2	21 ± 3	21 ± 3
Side-to-side PCS (%)	100	85	93
Mean intraoperative blood transfusions (U)	4.5*	3.5	2.1
Mean operative time (hr)	4.5*	4.2	3.1

*P <0.01.

PVT had some preoperative findings that were significantly different from those in patients without PVT (P <0.01) and indicated the presence of more advanced liver disease. The incidences of ascites (75%) and severe muscle wasting (52%) were higher in the patients with PVT, and significantly more of them (52%) were in Child's class C.

Table II shows the results of preoperative diagnostic studies in patients with and without PVT. All patients had esophageal or gastric varices and no other mucosal lesion that could account for bleeding. Almost all patients (94%) with PVT had very large varices; the incidence of varices of very large size was significantly higher (P <0.01) when PVT was present than when PVT was absent. A wedge liver biopsy obtained at operation showed cirrhosis of the liver in all patients, usually considered to be of the alcoholic type. However, it is likely that some alcoholic patients had posthepatitic cirrhosis rather than alcoholic cirrhosis, since results of serologic tests for hepatitis were not available in the early part of the study. In addition to more frequent varices of very large size, patients with PVT, compared to those without PVT, had significantly higher (P <0.01) incidences of the hyperdynamic cardiovascular state with a cardiac output of 6 L/min or higher and severe hypersplenism with a platelet count less than 50,000/mm^3.

Operative Findings

Table III presents the operative findings in patients with and without PVT. All patients had portal hypertension with a PV-IVC pressure gradient that averaged 285 mm saline solution in patients with PVT,

Table IV. Survival rates of patients with and without portal vein thrombosis following portacaval shunt

Survival	Emergency PCS		Elective PCS	
	With PVT (n = 65) (%)	Without PVT (n = 335) (%)	With PVT (n = 20) (%)	Without PVT (n = 880) (%)
Operative (30 days)	69	73	95	98.6
1 year	66	65	90	95
5 years	65	61	70	71
10-year actuarial	55	52	65	65
15-year actuarial	51	45	60	61

which was not significantly different from the pressure gradient in patients without PVT. PCS reduced the mean PV-IVC pressure gradient to 23 mm saline solution in patients with PVT, which is similar to the marked pressure reduction in patients without PVT. All patients with PVT underwent a side-to-side PCS.

Because of the technical operative maneuvers involved in phlebothrombectomy, operative blood loss, requirements for intraoperative blood transfusions, and time required to perform the operation were somewhat greater in patients with PVT compared to those without PVT ($P < 0.01$). However, in patients with PVT the requirement for blood transfusion during surgery averaged only 4.5 units and the mean duration of the operation was only 4.5 hours.

Control of Bleeding

PCS promptly stopped variceal bleeding in all patients who underwent emergency PCS. Permanent prevention of recurrent variceal bleeding or bleeding from any cause related to portal hypertension was successful in 95% of patients with PVT, 99% of patients without PVT who underwent emergency PCS, and 99.7% of patients without PVT who underwent elective PCS. In four patients with PVT, all of whom had occluded shunts, recurrent bleeding developed.

Survival

Table IV lists the survival rates following PCS for patients with and without PVT in each study group, and Fig. 1 presents the 15-year Kaplan-Meier survival curves. There were no significant differences in survival at any time interval between patients with and without PVT. In the emergency PCS group, which included unselected patients with acute variceal hemorrhage, 69% of those with PVT and 73% of those without PVT left the hospital alive. Subsequently the 1-, 5-, 10-, and 15-year survival rates were similar in

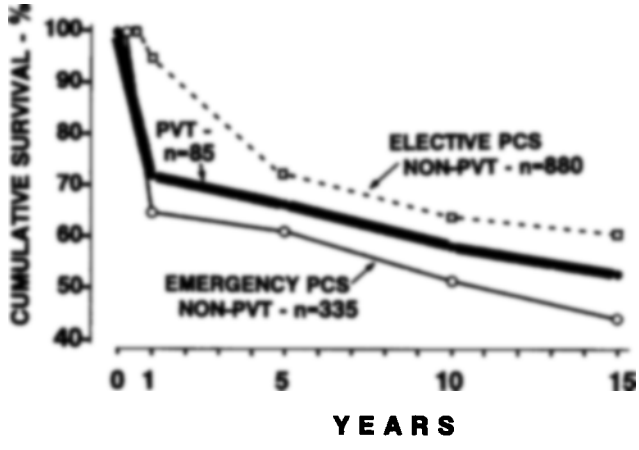

Fig. 1. Fifteen-year Kaplan-Meier survival curves after portacaval shunt *(PCS)* in patients with and without portal vein thrombosis *(PVT)*.

patients with and without PVT. After 15 years, 51% of the patients with cirrhosis who underwent phlebothrombectomy and emergency PCS were alive. In the elective PCS group, which involved highly selected patients, 95% of those with PVT and 98.6% of those without PVT left the hospital alive. Survival rates after 15 years in the elective shunt group were 60% of those with PVT and 61% of those without PVT. Long-term survival rates of the highly selected patients who underwent elective PCS were only 9% to 16% greater than those of the unselected patients, "all comers" included, who underwent emergency PCS.

Portal Systemic Encephalopathy

Before the operation, PSE was found on initial contact, was documented in the past medical records, or was noted at both times in 40% of patients with PVT and 33% of patients without PVT. During fol-

low-up a concerted effort was made at each clinic visit to identify PSE by evaluation of mental status, testing for asterixis, use of a series of timed number connection tests, and measurement of arterial blood ammonia. Recurrent PSE that required dietary protein restriction of less than 60 g/day, chronic therapy with lactulose or neomycin, and hospitalization one or more times was noted in 9% of patients with PVT, 9% of patients without PVT who underwent emergency PCS, and 7% of patients without PVT who had undergone elective PCS. The low incidence of PSE is consistent with our past experience and has been discussed at length previously.[1,7] Patients who abstained from alcohol and adhered to simple dietary instructions limiting protein intake to 60 g/day had a negligible incidence of PSE.

Quality of Life

Table V presents data on quality of life 5 years after PCS in patients with and without PVT. The low incidence of PSE has already been discussed. Occlusion of the PCS was demonstrated by yearly angiographic studies with pressure measurements, Doppler duplex ultrasonography, or both in four patients in the group with PVT, one patient without PVT in the emergency PCS group, and three patients without PVT in the elective PCS group. PCS patency at 5 years was 93%, 99.6%, and 99.7%, respectively. The infrequency of recurrent variceal bleeding has been discussed previously; it occurred in the small number of patients with occlusion of the PCS. Abstinence from alcohol throughout the 5-year period occurred in 61% of patients with PVT and in 63% to 64% of patients without PVT. Long-term survival rates were significantly higher in patients who abstained from alcohol. Continued heavy use of alcohol was responsible for most late deaths. Results of liver function tests 5 years after PCS, compared with findings before the operation, showed improvement in 68% of patients in the group with PVT, 62% without PVT in the emergency PCS group, and 75% without PVT in the elective PCS group. Excluding patients who were 65 years of age or older and who were classified as retired, 52% of patients in the group with PVT were gainfully employed or engaged in full-time housekeeping for part or all of the initial 5-year follow-up period. Corresponding frequencies in patients without PVT were 56% in the emergency PCS group and 64% in the elective PCS group.

DISCUSSION

It should be emphasized that all patients with PVT in the present study had well-organized thrombus in the main trunk of the PV, usually extending from the junction of the PV and splenic vein below to the bifurcation of the PV above. Not included in this study were patients with fresh clot in the PV, which commonly results from traction on the PV during operative dissection, thrombosis confined to the right or left PV branches, and intrahepatic PV thrombi.

The true incidence of PVT in cirrhosis is not known. In selected populations of patients the reported incidence has ranged from 0.6% in an autopsy study[11] to 21% in a study of patients examined by angiography or at laparotomy,[12] with numerous incidences between those extremes.[13-19] Several reports of

Table V. Quality of life at 5 years in patients with and without portal vein thrombosis

		Without PVT	
	With PVT (n = 85) (%)	Emergency PCS (n = 335) (%)	Elective PCS (n = 880) (%)
Shunt patency	93	99.6	99.7
Variceal rebleeding	5	1	0.3
Portal systemic encephalopathy			
Pre-shunt	40	33	33
Post-shunt recurrent	9	9	7
Alcohol abstinence	61	63	64
Improved liver function	68	62	75
Work status			
Retired because of age	14	20	12
Employed or housekeeping	52	56	64

patients with cirrhosis undergoing orthotopic liver transplantation have described an incidence of PVT ranging from 9% to 19%.[20-22] The incidence in our large series of cirrhotic patients with variceal hemorrhage ranged from 16% in the unselected acutely bleeding patients to 2% in the highly selected patients who underwent elective operation. It is clear that PVT in cirrhosis with variceal hemorrhage is not uncommon.

Many conditions in adults other than cirrhosis lead to PVT.[23] Common causes include malignant neoplasms, particularly pancreatic carcinoma and hepatocellular carcinoma, intra-abdominal infections, pancreatitis, myeloproliferative diseases, hypercoagulable states, abdominal trauma, splenectomy, and various abdominal operations involving or near the portal system. It has been proposed that endoscopic sclerotherapy of esophageal varices is associated with PVT, but our experience and recent studies[24,25] have failed to demonstrate such an association. None of our patients had any of the conditions, other than cirrhosis, that lead to PVT. Some of our patients treated in the final decade of our studies had undergone endoscopic sclerotherapy at some time prior to their admission for PCS, but there is nothing to indicate that the incidence of PVT in these patients was different from that in patients who did not undergo variceal sclerotherapy. It is believed, but has not been proved, that PVT in patients with cirrhosis, such as those in the present study, results from obstruction of portal blood flow within the liver with consequent stagnation and clotting of blood in the PV.

Patients with PVT in the present study had more advanced cirrhosis as a group than patients without PVT. Compared to patients without PVT, more of those with PVT were in Child's class C, and they had higher incidences of ascites, severe muscle wasting, varices of very large size, the hyperdynamic cardiovascular state, and severe hypersplenism. Nevertheless, there was nothing unusual about their clinical characteristics, and there were no clinical or diagnostic data that would cause one to suspect the presence of PVT.

It is possible to make a diagnosis of PVT by means of various radiologic techniques, including ultrasonography, particularly with the Doppler duplex technique, contrast-enhanced CT, MRI, and splanchnic arteriography with indirect portography. We did not use any of these techniques because we found it possible to manage PVT effectively when it was discovered at operation. Moreover, these procedures are time consuming, particularly in acutely bleeding patients, and they are costly. In all of the patients in the present study, the diagnosis of PVT was made during the PCS operation. Nevertheless, an argument could be made for routinely using a noninvasive technique such as ultrasonography in patients referred for elective PCS.

PVT has often been considered a contraindication to both PCS and orthotopic liver transplantation. The most important result of the present study is the demonstration that it is usually possible to extract organized thrombus from the PV and then perform a PCS with an outcome equivalent to that achieved in patients without PVT. Belli et al.[18] came to a similar conclusion in the only other study of phlebothrombectomy of PVT followed by portosystemic shunt in a sizable number of patients. Furthermore, successful phlebothrombectomy of the PV has been reported with increasing frequency in patients undergoing orthotopic liver transplantation.[20-22]

The long-term PCS patency rate of 93% in the present series of patients with PVT is noteworthy. We believe it resulted from the restoration of brisk portal blood flow across the large-diameter portacaval anastomosis. The patients in the present study were placed on a daily oral tablet (325 mg) of acetylsalicylic acid for life. Anticoagulants such as heparin and warfarin sodium were not used because of concern about postoperative bleeding, but an argument might be made for their use.

Quality of life was satisfactory in most patients in the PVT group who survived PCS for a year or more and was reflected in a low incidence of recurrent PSE, freedom from gastrointestinal bleeding, a substantial rate of abstinence from alcohol, improvement in liver function in 68%, and improvement in Child's risk class in 70% of patients. Quality of life was similar in patients with and without PVT. One half of the patients in the PVT group who were not of retirement age were gainfully employed or doing full-time housekeeping for part or all of the first 5-year follow-up period. Patients who survived PCS for 6 months had a good chance of prolonged survival. Not surprisingly, resumption of alcohol imbibition played a major role in late death.

The results of the present study demonstrate that phlebothrombectomy combined with direct side-to-side PCS can be accomplished successfully in almost all patients with cirrhosis and variceal hemorrhage who have organized thrombus in the main trunk of the PV. In such patients PCS eliminates the high PV-IVC gradient, carries a high long-term patency rate, and provides lifelong protection against recurrent variceal bleeding in almost all patients. The outcome is no different from that achieved with PCS in the absence of PVT and includes substantial long-term survival, a low incidence of PSE, improved liver function, and a life of good quality in many patients.

REFERENCES

1. Orloff MJ, Orloff MS, Orloff SL, Girard B. Three decades of experience with emergency portacaval shunt for acutely bleeding esophageal varices in 400 unselected patients with cirrhosis of the liver. J Am Coll Surg 1995;180:257-272.
2. Orloff MJ. Emergency portacaval shunt: A comparative study of shunt, varix ligation, and nonsurgical treatment of bleeding esophageal varices in unselected patients with cirrhosis. Ann Surg 1967;166:456-478.
3. Orloff MJ, Charters AC III, Chandler JG, Condon JK, Grambort DE, Moddaferi TR, Levin SE, Brown NB, Sviokla SC, Knox DG. Portacaval shunt as emergency procedure in unselected patients with alcoholic cirrhosis. Surg Gynecol Obstet 1975;141:59-68.
4. Orloff MJ, Bell RH Jr, Hyde PV, Skivolocki WP. Long-term results of emergency portacaval shunt for bleeding esophageal varices in unselected patients with alcohol cirrhosis. Ann Surg 1980;192:325-340.
5. Orloff MJ, Bell RH Jr. Long-term survival after emergency portacaval shunting for bleeding varices in patients with alcoholic cirrhosis. Am J Surg 1986;151:176-183.
6. Orloff MJ, Orloff MS, Rambotti M, Girard B. Is portal-systemic shunt worthwhile in Child's class C cirrhosis? Ann Surg 1992;216:256-268.
7. Orloff MJ, Bell RH Jr, Orloff MS, Hardison WG, Greenburg AG. Prospective randomized trial of emergency portacaval shunt and emergency medical therapy in unselected cirrhotic patients with bleeding varices. Hepatology 1994;20:863-872.
8. Child CG III, Turcotte JG. Surgery and portal hypertension. In Child CG III, ed. The Liver and Portal Hypertension. Philadelphia: WB Saunders, 1964, pp 1-85.
9. Campbell DP, Parker DE, Anagnostopoulos CE. Survival prediction in portacaval shunt: A computerized statistical analysis. Am J Surg 1973;126:748-751.
10. Orloff MJ, Orloff MS. Bleeding oesophageal and gastric varices: Shunts. In Jamieson GG, Debas HT, eds. Rob & Smith's Operative Surgery: Surgery of the Upper Gastrointestinal Tract, 4th ed. London: Chapman & Hall, 1994, pp 493-515.
11. Okuda K, Ohnishi K, Kimura K, Matsutani S, Sumida M, Goto N, Musha H, Takashi M, Suzuki N, Shinagawa T, Suzuki N, Ohtsuki T, Arakawa M, Nakashima T. Incidence of portal vein thrombosis in liver cirrhosis. Gastroenterology 1985;89:279-286.
12. Sarfeh IJ. Portal vein thrombosis associated with cirrhosis. Arch Surg 1979;114:902-905.
13. Hunt AH, Wittard BR. Thrombosis of the portal vein in hepatic cirrhosis. Lancet 1954;1:281-284.
14. Dye WS, David D, Julian OC. Successful treatment of portal vein thrombosis associated with intrahepatic obstruction. Arch Surg 1960;80:876-882.
15. Coomaraswamy RP, Del Guercio LRM, Miller H, State D, Elkin M. Splenoportography and portal vein thrombosis in patients with cirrhosis of the liver. Surg Gynecol Obstet 1964;118:560-566.
16. Ranganathan N, Burch CE. Incidence of thrombosis in portal venous stasis and hypertension. South Med J 1969;62:302-306.
17. Sicot C, Sakellaridis D, Rueff B, Maillard JN, Benhamou JP. Portal vein thrombosis in intrahepatic block. Minn Med 1971;54:87-90.
18. Belli L, Sansalone CV, Aseni P, Romani F, Rondinara G. Portal thrombosis in cirrhotics. Ann Surg 1986;203:286-291.
19. Monarca A, Natangelo R, Tavani E, Azzolini V. Cirrhosis and portal vein thrombosis [letter]. Gastroenterology 1986; 90:509.
20. Nonami T, Yokoyama I, Iwatsuki S, Starzl TE. The incidence of portal vein thrombosis at liver transplantation. Hepatology 1992;16:1195-1198.
21. Cherqui D, Duvoux C, Rahmouni A, Rotman N, Dhumeaux D, Julien M, Fagniez PL. Orthotopic liver transplantation in the presence of partial or total portal vein thrombosis: Problems in diagnosis and management. World J Surg 1983; 17:669-674.
22. Davidson BR, Gibson M, Dick R, Burroughs A, Rolles K. Incidence, risk factors, management, and outcome of portal vein abnormalities at orthotopic liver transplantation. Transplantation 1994;57:1174-1177.
23. Cohen J, Edelman RR, Chopra S. Portal vein thrombosis: A review. Am J Med 1992;92:173-182.
24. Kawasaki S, Henderson JM, Riepe SP, Brooks WS, Hertzler G. Endoscopic variceal sclerosis does not increase the risk of portal venous thrombosis. Gastroenterology 1992;102:206-215.
25. Politoske D, Ralls P, Korula J. Portal vein thrombosis following endoscopic variceal sclerotherapy. Prospective controlled comparison in patients with cirrhosis. Dig Dis Sci 1996; 41:185-190.

Discussion

Dr. H. Orozco (Mexico City, Mexico). My own experience includes caring for more than 800 patients with portal hypertension; 11 of them were found to have cirrhosis and PVT.

How is it possible to perform emergency PCS in Child's class C patients, given the condition of their veins, and keep them alive? Why do you continue to perform these PCS procedures that direct the portal blood flow away from the liver?

Dr. M.J. Orloff. In patients with what is essentially cavernous transformation of the portal vein, it is not possible to perform a PCS. In fact, most patients with PVT have thrombus confined to the main trunk of the portal vein and the thrombus can be readily removed. The results speak for themselves. The notion that maintaining flow toward the liver is an essential part of life is just not what these data would support, so I stand on the results rather than the unsupported opinion you have expressed.

Dr. W. Meyers (Worcester, Mass.). If you were to grade the degree of difficulty of these operations on a scale of 1 to 10, with 1 being relatively easy and 10 being relatively difficult, what would be the distribution?

Dr. Orloff. I think it varies greatly, and not because of the PVT. It varies because of all the circumstances that are encountered in patients who are bleeding from esophageal varices. Generally speaking, the emergency operations are the most difficult; usually, operations in Child's class C patients are more difficult than the elective operations, but I

think there is a whole spectrum of difficulty and it is not related to the PVT.

Dr. L. Rikkers (Madison, Wis.). Could you speculate as to what you believe is the pathogenesis of the PVT in these patients? Is it secondary to portal venostasis? Were any of these thromboses detected preoperatively? I know that your patients are rapidly brought to surgery, but if you knew that a patient had a complete PVT preoperatively, would you take a different approach? How many of these were partial and how many were complete thrombosis? We have found patients in both the pediatric and adult populations with cavernomatous transformation to be ideal candidates for distal splenorenal shunt since the splenic vein is open; these patients have good portal perfusion through hepatopetal collateral vessels and portal perfusion tends to be retained indefinitely because these patients have a low-resistance liver. Would you favor a distal splenorenal shunt in noncirrhotic patients with PVT?

Dr. Orloff. Regarding the pathogenesis, of course that is speculative. The speculation, which did not originate with our group, is there is so much stasis in the portal vein as a result of the intrahepatic obstruction, sometimes to the degree where there is no forward or backward flow, and that is what leads to the thrombosis. Although this is speculative, there really are no other data to implicate another factor. As far as preoperative detection, I think we would emphasize that, particularly in patients who undergo emergency shunts, it really is not worthwhile to perform angiography or noninvasive imaging studies because it is usually possible to remove the thrombus. For 8 years we did perform angiography routinely in all of our emergency shunt patients, but we stopped because it was time consuming and it was not really an effective means of separating patients. In my opinion Doppler duplex ultrasound studies would be worthwhile in patients undergoing elective shunts. I cannot answer the question about partial vs. complete obstruction. The thrombus is visible in the vein, but it is not possible by "eyeballing" it to determine how much blood, if any, is getting past the thrombus. So I really cannot answer that question. With regard to patients with extrahepatic portal hypertension with cavernomatous transformation of the portal vein, as we all know, that is a totally different disease. It is a disease in which we have been interested. It is not possible to perform a phlebothrombectomy in those patients. We have, within the past year or so, reported a series of 162 patients with PVT, and our standard practice has been to perform either a splenorenal shunt or a mesocaval shunt using a direct anastomosis rather than a synthetic graft. It is acceptable to perform a distal splenorenal shunt; it is just not the operation that we have chosen. The results are uniformly good in young persons who have extrahepatic portal obstruction, provided they undergo decompression.

Dr. I.J. Sarfeh (Long Beach, Calif.). We published our series in the late 1970s with far more dismal results than yours. Our problem was that often the thrombus extended into the distal portion of the splenic vein just at the confluence, and we were unable to remove the thrombus. In some of our patients, in order to control variceal hemorrhage, we had to reoperate and perform a splenectomy. Did you have that problem and did you use postoperative angiography to see if the portal venous system had been completely cleared?

Dr. Orloff. I did read your article very carefully and found some differences between your study and ours. For one thing, yours was a retrospective chart review. Your results really were not very encouraging. Had I read your report before we undertook our prospective study, I think we probably would not have done what we did because of your discouraging findings. Fortunately I did not read it until afterward. We followed all of these patients by means of yearly angiography and/or Doppler duplex ultrasonography to determine shunt patency, so the patency figures that I am reporting are very accurate long-term patency figures. We did not perform splenectomy in a single patient in this series. I believe that splenectomy in patients with hypersplenism is really unnecessary if portal systemic decompression is employed. The hypersplenism is resolved and the platelet count rises promptly after portal systemic decompression.

Role of Protein Kinase A Pathway in Epidermal Growth Factor–Induced Liver Cell Repair

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To gain a better understanding of the mechanisms that control the repair process in the injured liver, the actions of epidermal growth factor (EGF) and protein kinase A (PKA) were studied. Normal rat liver cells (clone 9) were grown to confluence. Standardized excisional wounds were made with a razor blade. The extent of hepatocyte migration into the wound was measured and determined at specific time intervals using a computerized digital analyzing system. Immunostaining of F-actin was performed with a fluorescein-labeled phalloidin. EGF significantly stimulated liver cell migration, whereas specific EGF-neutralizing antibody inhibited the EGF-induced migration. Agents that activate PKA at different stages of the PKA activation pathway, including 3-isobutyl-1-methylxanthine (IBMX), forskolin, and cholera toxin, inhibited EGF-induced migration. EGF triggered formation of actin stress fibers. PKA-activating agents inhibited actin stress fiber formation and stretching of cells at the wound margin. The following conclusions were drawn: (1) In excisional wounds of hepatocyte monolayers, both EGF and PKA exert action on actin microfilaments, which are stretched by EGF and inhibited by PKA; (2) the enhanced repair of wounded hepatocyte monolayers by EGF is blocked by activation of the PKA pathway at various levels; and (3) these actions of EGF and PKA indicate their important regulatory roles in controlling the rate of hepatocyte migration and restitution following the creation of excisional wounds. (J GASTROINTEST SURG 1997;1:132-137.)

Cell migration is an important initial step in the repair of the injured liver, an organ increasingly subjected to both surgical and other forms of trauma. Although extensive studies have demonstrated that several growth factors and hormones play a role in liver restitution, the intracellular processes involved in its regulation are not well understood.[1-8] Cells need to communicate with one another to control their growth and division and coordinate their functions. Cells secrete growth factors and hormones that provide a signal to other cells. Second messenger pathways play an important role in this process after growth factors or hormones bind to their specific receptors. However, the role of protein kinase A (PKA) on liver cell migration is unclear. Understanding the intracellular signaling processes involved in the regulation of liver cell migration is key to developing strategies both physiologically and pharmacologically that modulate liver restitution. Therefore we examined the interactions of epidermal growth factor (EGF)—a major stimulator of hepatocyte migration—and second messenger pathways in the regulation of liver epithelial cell migration in wounded hepatocyte tissue cultures.

MATERIAL AND METHODS

Cell Culture

Liver epithelial (clone 9) cells established from a normal liver taken from a 4-week-old Sprague-Dawley male rat in 1968 were obtained from American Type Culture Collection (Rockville, Md.) at passage 16. The stock cultures were grown in an atmosphere of 5% carbon dioxide at 37° C in a culture medium composed of F12K Kaighn's modification (Life Technologies, Inc., Gaithersburg, Md.) with 1260 mg/ml

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glucose, 292 mg/ml glutamine, 1000 U/ml penicillin G, 2.5 μg/ml amphotericin B, 1000 μg/ml streptomycin, and 10% fetal bovine serum (FBS). The culture medium was changed every 2 days. The cells were subcultured by partial digestion with 0.5 g/L trypsin and 0.2 g/L ethylenediaminetetraacetic acid in Hanks' balanced salt solution. Normal rat liver cells were detached from stock cultures by trypsin digestion, washed once by centrifugation, resuspended, and subcultured in 20 ml medium in cultured flasks at a concentration of 5×10^5 cells/ml.

Migration Studies

The newly plated rat liver cells were grown to confluence (4 to 5 days post plating) on 6-well plates. The cells were rinsed with F12K solution. Standardized excisional wounds (25 mm) were made by scraping with a single-edge razor blade as previously described.[9,10] After scraping, "wounded" monolayers were washed with F12K solution. Subsequently EGF (0 to 120 ng/ml), anti-EGF antibody (0 to 5 mg/ml with EGF 30 ng/ml), 3-isobutyl-1-methylxanthine (IBMX; 0 to 1 mmol/L with EGF 30 ng/ml), forskolin (0 to 150 mmol/L with EGF 30 ng/ml), or cholera toxin (0 to 100 ng/ml with EGF 30 ng/ml) was added. Wounded monolayers were incubated at 37° C for 24 hours. At the end of the 24-hour incubation period, the incubation solution was removed, and the cells were fixed in methanol and stained with Mayer's hematoxylin staining solution. To verify the contribution of DNA synthesis and proliferation to the observed liver cell migration, the labeling index of bromodeoxyuridine (BrdU) and the mitotic activity of liver cells were assessed. BrdU, 0.03 g/ml, was added 1 hour before immunostaining to confluent liver cells just after wounding on collagen I–coated glass coverslips, then fixed in a 3.7% formaldehyde solution in phosphate-buffered saline (PBS) for 20 minutes, and washed with PBS for 10 minutes each three times. Then liver cell monolayers were permeabilized in acetone at −20° C for 5 minutes and stained as previously described.[11] The labeling index of BrdU and the number of dividing cells were assessed by counting 1000 cells under the microscope. The labeling index of BrdU was determined by sequential measurement of the percentage of BrdU-positive cells in the migrating region incubated with medium alone and EGF 30 ng/ml every 6 hours until 24 hours following wound formation. The mitotic activity was determined by sequential measurement of the percentage of dividing cells (nuclear division) incubated with medium alone and EGF 30 ng/ml in both the migrating and nonmigrating regions under ×100 magnification every 2 hours until 26 hours following formation of the wound.

To minimize the effect of cell proliferation and to eliminate the contribution of various serum factors present in FBS on cell migration, all of the migration experiments that follow were carried out in F12K buffer solution without FBS.

Liver epithelial cell migration was measured by means of a computerized image analyzer system composed of a binocular microscope (TMS-F No. 2, Nikon, Tokyo, Japan). This was attached to a Macintosh computer using the NIH Image Program version 1.58 (National Institutes of Health, Bethesda, Md.) to measure the area traveled by the migrating cells. Five measurements were taken from each well. To eliminate potential observer bias, the plates were number coded and read by a "blinded" observer who had no knowledge of the codes and was not involved in the experiments. All of the experiments were repeated three to six times to ensure reproducibility.

Immunofluorescent Labeling of Cytoskeletal Elements

Liver cells were grown to confluence on collagen I–coated glass coverslips and wounded as previously described. Immunostaining for actin was performed as previously described[12] using fluorescein-labeled phalloidin, and mounted in a 1:1 solution of PBS and glycerol. The distribution of actin microfilaments was examined under a Nikon microscope with epifluorescence.

Statistical Analysis

Results were expressed as mean ± standard error of the mean. Statistical significance of differences between mean values was assessed by means of Student's *t* test for unpaired data. All reported significance levels represent two-tailed *P* values.

RESULTS

Effect of EGF

The addition of varying doses of EGF resulted in a concentration-dependent increase in migration of rat liver cells at the wound margin (Fig. 1, *A*). At 30 ng/ml EGF concentration, liver cell migration plateaued and increasing the EGF concentration to greater than 30 ng/ml did not produce a further increase in liver cell migration. Anti-EGF produced marked inhibition of EGF-induced cell migration in a concentration-dependent manner.

Micrographs of the liver cell monolayer shortly after wound formation and after 24 hours of migration are shown in Fig. 2. There is a sharp demarcation of

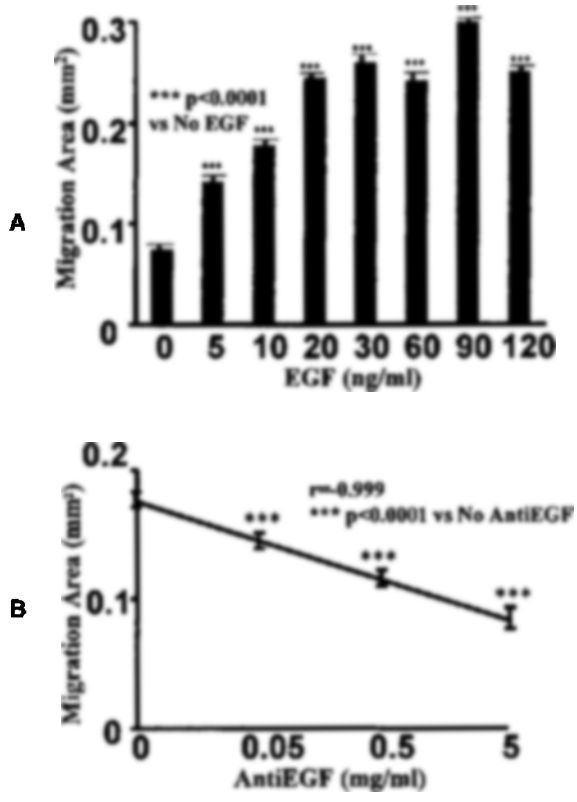

Fig. 1. A, Effect of varying concentrations of EGF on liver cell migration. Confluent liver cell monolayers on plastic plates were wounded by scraping with a single-edge razor blade (as described in Methods) and incubated in F12K buffer solution containing various concentrations of EGF. The total area (mm^2) of migration (mean ± SEM) between the 3.8 mm width was measured using a computerized image analysis system (n = 8). **B,** Effect of neutralizing anti-EGF antibody on EGF-induced liver cell migration on plastic plates. EGF 30 ng/ml and varying concentrations of anti-EGF antibody were added following wound formation (n = 4).

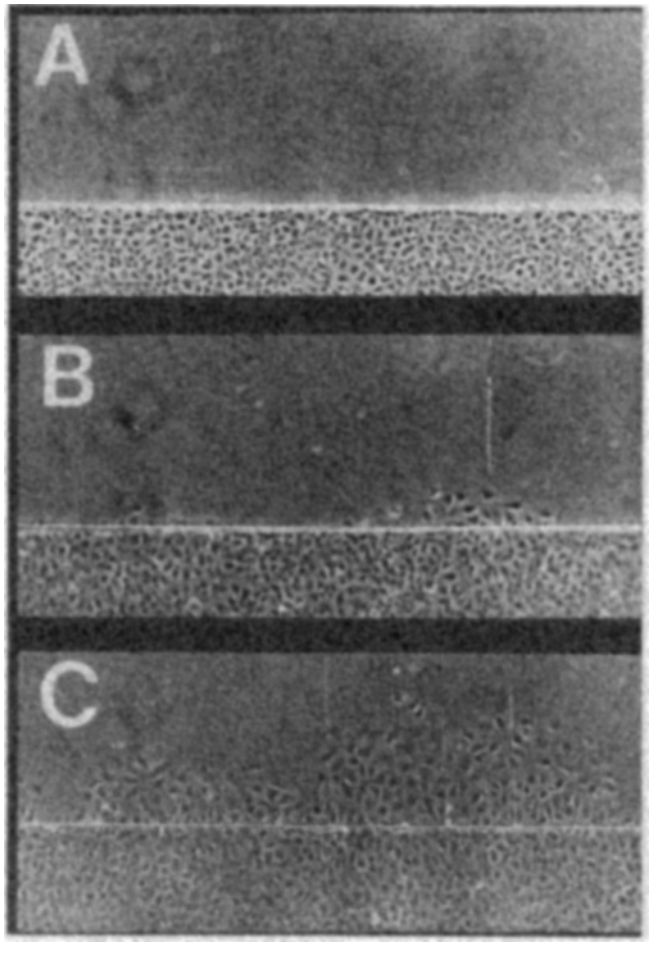

Fig. 2. Photomicrograph of liver epithelial cell formation immediately after wound formation **(A)**, 24 hours after wound formation with F12K medium **(B)**, and 24 hours after wound formation treated with EGF 30 ng/ml **(C)**. (Original magnification ×400.)

the wound site following razor blade scraping as identified by the linear scratch mark. This line was used as the reference point for the measurement of liver cell migration. Immediately following wound formation, the cells below the margin of the wound appear normal and there is no cell migration (see Fig. 2, *A*). The cells are polygonal in shape and tightly packed. By 24 hours much of the wound is covered by the confluent epithelial layer (see Fig. 2, *B* and *C*). As shown in Fig. 2, *C*, the cells at the migration front are more spread out and have a much larger cross-sectional area than cells far away from the migration front or their "native" confluent state.

The results of the labeling index of BrdU and the mitotic activity of liver cells are shown in Table I. The labeling index of BrdU began to increase at 18 hours after wounding and achieved a 10-fold increase over the labeling index at 0 hour. The number of dividing cells increased but the range of increase was less than 0.4%. Thus the cells began to synthesize DNA at 18 hours after wounding, but division of cells was negligible until 26 hours after wounding.

Effect of PKA Activators on Liver Cell Migration

Both IBMX and forskolin produced a dose-dependent inhibition of liver cell migration (Fig. 3, *A* and *B*). Cholera toxin also produced a marked inhibition of cell migration (Fig. 3, *C*).

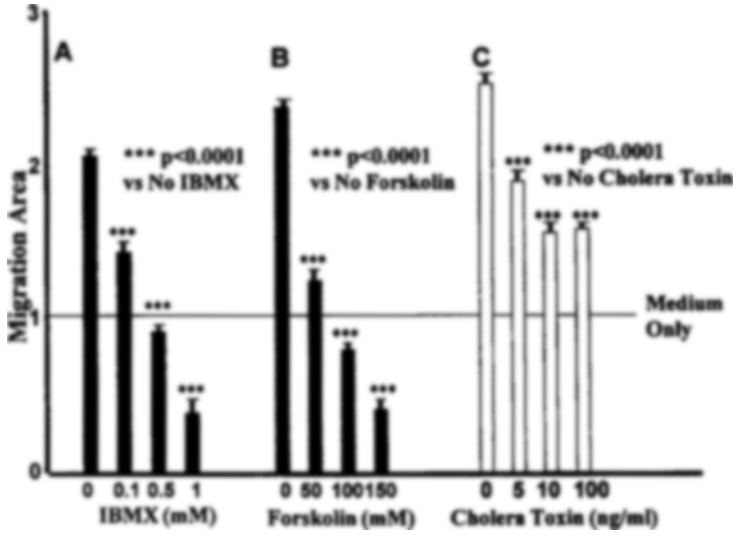

Fig. 3. The effects of 3-isobutyl-1-methylxanthine (IBMX) **(A)**, forskolin **(B)**, and cholera toxin **(C)** on EGF-induced liver cell migration. The total area of migration is expressed as fractional migration (mean ± SEM) of control monolayers. The fractional migration of control monolayers was assigned a value of 1.0.

Table I. Labeling index of BrdU and number of dividing cells

	BrdU (%)		Dividing cells (%)			
	Medium	EGF	Medium		EGF	
Time (hr)	Margin	Margin	Margin	Outside margin	Margin	Outside margin
0	1.13	1.13	0.55	0.38	0.54	0.46
2	—	—	0.68	0.56	0.47	0.38
4	—	—	0.54	0.56	0.62	0.47
6	1.37	1.15		0.20	0.39	0.84
8	—	—	0.48			
10	—	—	0.52	0.59	0.65	0.41
12	1.26	2.77	0.59	0.60	0.40	0.29
14	—	—	0.35	0.50	0.47	0.35
16	—	—	0.62	0.35	0.66	0.64
18	1.63	4.76	0.70	0.48	0.67	0.50
20	—	—	0.53	0.44	1.2	0.32
22	—	—	0.29	0.44	0.20	0.35
24	4.99	10.33	0.56	0.47	0.64	0.72
26	—	—	0.47	0.49	1.2	0.75

Role of Actin Microfilaments on Liver Cell Migration

Following wounding, EGF treatment caused an increase in the assembly of actin stress fibers with marked stretching of liver cells at the margin of the wound front (Fig. 4, A and *B*). IBMX almost completely inhibited formation of actin stress fibers and stretching of the cells at the migration front (Fig. 4, *C*).

DISCUSSION

This study demonstrates that liver cell migration is regulated by several factors including EGF, the second messenger pathway (protein kinase A), and cytoskeletal elements (actin microfilaments). We found that liver cell migration and proliferation are both stimulated by EGF. However, cell division did not take place until 26 hours after wounding, indicating

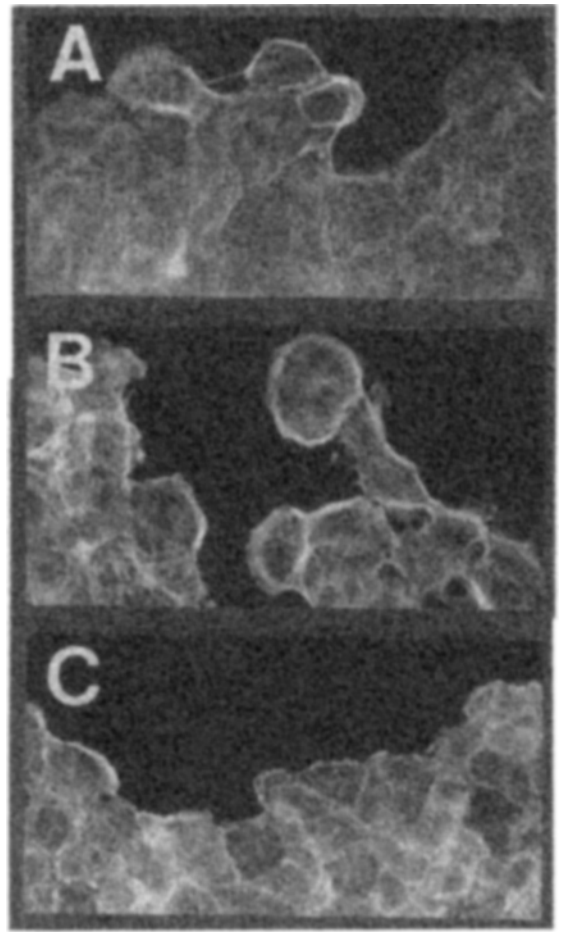

Fig. 4. Immunofluorescent localization of liver cell actin microfilaments. Immunofluorescent localization of actin microfilaments in controls **(A)**, EGF 30 ng/ml **(B)**, and IBMX 1 mmol/L **(C)**, treated monolayers for 24 hours. (Original magnification ×400.)

that liver cell migration alone is the initial response to hepatic wounding followed later by cell division.

Some water-soluble signaling molecules (growth factors or hormones) bind to their specific receptor on the surface of cells first. The receptor then stimulates G-protein, which activates adenylate cyclase. As a result the second messenger—cyclic adenosine monophosphate (cAMP)—is synthesized from adenosine triphosphate by adenylate cyclase and cAMP activates PKA. In our study hepatocyte migration was inhibited by the activation of the PKA pathway. This was evidenced in the following three ways: (1) IBMX, a potent phosphodiesterase inhibitor that suppresses degradation of intracellular cAMP, inhibits EGF-induced liver cell migration. This finding suggests that liver cell migration is restricted by activation of PKA. (2) Forskolin, which activates adenylate cyclase and increases intracellular cAMP, also produced a dose-dependent inhibition of liver cell migration. (3) Cholera toxin, an enzyme that activates stimulatory G-protein, also inhibits hepatocyte migration, further demonstrating the suppressive role of adenylate cyclase activation.

Mobility of cells is influenced to a great extent by F-actin.[12] In this study EGF treatment caused an increase in the assembly of actin stress fibers with marked stretching of liver cells at the margin of the wound. This suggests that EGF increased the mobility of actin microfilaments of liver cells after wounding. In contradistinction, activation of PKA inhibited actin stress fiber formation and stretching of cells at the wound front.

CONCLUSION

We conclude that both EGF and PKA exert actions on actin microfilaments, which are stretched by EGF and inhibited by PKA in excisional wounds of liver cells. Th enhanced repair of wounded hepatocyte monolayers by EGF is blocked by activation of the PKA pathway at various levels. These actions of EGF and PKA indicate their important regulatory roles on controlling the rate of liver cell migration and restitution following creation of incisional wounds.

REFERENCES

1. Bade EG, Feindler S. Liver epithelial cell migration induced by epidermal growth factor or transforming growth factor-a is associated with change in the gene expression of secreted proteins. In Vitro Cell Dev Biol 1988;24:149-154.
2. Tomiya T, Fujiwara K. Serum levels of transforming growth factor-α in patients after partial hepatectomy as determined with an enzyme-linked immunosorbent assay. Hepatology 1993;18:304-308.
3. Michalopoulos GK, Zarnegav R. Hepatocyte growth factor. Hepatology 1992;15:149-155.
4. Fujiwara K, Nagoshi S, Ohno A, Hirata K, Ohta Y, Mochida S, Tomiya T, Higashio K, Kurokawa K. Stimulation of liver growth by exogenous human hepatocyte growth factor in normal and partially hepatectomized rats. Hepatology 1991; 14:901-905.
5. Nakamura T, Teramoto H, Ichihara A. Partial purification and characterization of a growth factor from rat platelets in primary culture. Proc Natl Acad Sci USA 1986;83:6489-6493.
6. Strain AJ, McGuinness G, Rubin JS, Aaronson SA. Keratinocyte growth factor and fibroblast growth factor action on DNA synthesis in rat and human hepatocytes: Modulation by heparin. Exp Cell Res 1994;210:253-259.
7. Leffert HL, Koch KS, Moran T, Rubalcava B. Hormonal control of rat liver regeneration. Gastroenterology 1979;76: 1470-1482.

8. Massagure J, Blinderman LA, Czech MP. The high affinity insulin receptor mediates growth stimulation in rat hepatoma cells. J Biol Chem 1982;257:13958-13963.
9. Kvietys PR, Specian RD, Grisham MB, Tso P. Jejunal mucosal injury and restitution: Role of hydrolytic products of food digestion. Am J Physiol 1991;261:G384-G391.
10. MacCormack SA, Viar MJ, Johnson LR. Migration of IEC-6 cells: A model for mucosal healing. Am J Physiol 1992; 263:G422-G435.
11. Fujiwara Y, Tarnawski A, Fujiwara K, Arakawa T, Kobayashi K. Inhibitory effects of indomethacin on growth and proliferation of gastric carcinoma cells KATO III. J Physiol Pharmacol 1993;44:147-153.
12. Nusrat A, Delp C, Madara JL. Intestinal epithelial restitution. J Clin Invest 1992;89:1501-1511.x

Litigious Consequences of Open and Laparoscopic Biliary Surgical Mishaps

James G. Chandler, M.D., C. Randle Voyles, M.D., Tammy L. Floore, B.S.N., Lori A. Bartholomew, B.S.

Three hundred six injuries or complications coincident to 296 laparoscopic cholecystectomies were analyzed for the nature and extent of injuries and litigious outcomes that followed. The data were drawn from 31 member companies of the Physician Insurers Association of America, a trade association that initiated the study. The outcomes were compared to 261 contemporaneous open cholecystectomy claims. Biliary tract injuries were the most common, accounting for almost two thirds of all injuries. The spectrum of cases, originally selected for indemnity potential, reflected relative incidences in the medical literature. Laparoscopic injuries were significantly more severe, more likely to result in indemnity, and more apt to involve higher mean ± standard deviation dollar values ($160 ±154 × 10^3) to surviving claimants than injuries resulting from open procedures ($106 ± 122 × 10^3, $P = 0.01$). Injury recognition at the time of the original procedure had no discernible mitigating effect because 80% of recognized injuries required an additional operative procedure. Risk-aversive behavior should include paying particular attention to placement of the first port, more liberal use of the Hasson technique, placement of all other ports under direct vision, elimination of intraoperative anatomic uncertainty, programmed inspection of the abdomen before withdrawing the laparoscope, and acquiring sufficient knowledge of electrosurgical principles to ensure the safe use of this potentially dangerous modality. (J GASTROINTEST SURG 1997;1:138-145.)

Because of its phenomenal acceptance over the past 8 years, laparoscopic cholecystectomy has largely displaced open cholecystectomy, as well as lithotripsy and medical dissolution of gallstones. It has also spawned a large number of reports—42 in 1995 alone—that describe complications and injuries coincident to the performance of laparoscopic cholecystectomy. The best of these articles cover sizable multiinstitutional experiences and employed special measures to ensure reporting compliance, thereby providing reliable incidence estimates.[1-4] The next tier is characterized by less disciplined but huge case samples from a large-scale survey or comprehensive compilation, providing a broader overview.[5,6] Other articles report a skewed perspective, derived exclusively from referral centers, providing valuable information about details that are important to the success of remedial surgical procedures and sometimes conflicting inferences regarding what is happening on the front line.[7-10] Then there are the "master craftsman" series that set standards of excellence, best exemplified by the recent publication by Dubois et al.,[11] the progenitors of laparoscopic cholecystectomy. All of these articles have largely focused on bile duct injuries, which appear to have had a real and persistent increase, even though laparoscopic cholecystectomy is said to be generally safer in all other ways and is more convenient than open cholecystectomy.[1,3,5-7,12,13] Last, there are numerous small series and case reports that offer scant data and no evidence but raise vexatious questions about the true incidence of such things as trocar and Veress needle injuries,[14,15] CO_2 embolism,[16,17] or electrothermal bowel perforation.[18]

The results reported herein are based on claims data from 1989 to 1993 compiled by the Physician Insurers Association of America (PIAA), which is a trade association that is now supported by 53 physician- and dentist-owned member companies. An original analysis of these data was completed in 1994 and distributed to member companies and other interested organizations as a pamphlet entitled "Physician Insurers Association of America Laparoscopic Procedure Study."[19] The claims data have been revisited now that more claims have been settled, the nature of the precipitating in-

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juries has been better defined, and claims data have been collapsed into patient-specific data when appropriate. This article clearly belongs among the "skewed perspective" reports but, as such, it is uniquely revealing because its original case selection was based on plaintiff's bar appraisal of the likelihood that an indemnity payment might eventuate.

MATERIAL AND METHODS

In 1993 thirty-one insurance companies submitted 331 survey forms covering individual claims arising from laparoscopic cholecystectomies performed between January 1989 and June 1993. The survey was completed in August 1993, at which time only 30% of claims had been resolved. In addition to indemnity and demographic data, the forms documented the indication for the operation, performance of intraoperative cholangiography, description of the injury, whether or not the injury was recognized during the original procedure (defined by the patient having left the operating room), and a National Association of Insurance Commissioners (NAIC) severity index code.[20] The NAIC code is commonly accepted throughout the United States insurance industry as a measure of severity in personal injury cases. It takes into account both the nature of the original injury and the outcome, assigning code scores ranging from 1 to 9 (Table I).

In 1996 the PIAA requested that contributing member companies update the study's claims information, which more than doubled the number of claims with known resolution status. The association also permitted the authors to review the survey forms, deleting only the names of the reporting companies. Twenty-five of the original 331 forms were not available for review. The injury descriptions, occasional comments about cause, the injury site relative to the immediate operative field, and whether or not the injury was discovered during the original procedure, when taken together, allowed us to divide biliary injuries into three classes of severity and to define the causes of injury with minimal residual uncertainty. Although the 1994 PIAA analysis compared noncontemporaneous open cholecystectomies with laparoscopic procedures, sufficient data had now accumulated in their ongoing study of open cholecystectomies and other general surgical procedures to allow selection of a 1989 to 1993 open cholecystectomy cohort.

Data were analyzed using the Statistical Analysis System (Cary, N.C.). Chi-square analysis was used to compare proportions, and the relationship between outcome variables and various covariates was modeled using linear logistic regression analysis.[21]

Table I. NAIC severity index code

Injury/outcome	Code
Emotional injury only	1
Insignificant injury	2
Minor temporary injury	3
Major temporary injury	4
Minor permanent injury	5
Significant permanent injury	6
Major permanent injury	7
Quadriplegic, brain damage, lifelong care	8
Death	9

RESULTS

Demographics

Three hundred six laparoscopic claims originated from 296 claimants, and 261 open cholecystectomy claims stemmed from approximately 250 claimants. The mean age ± standard deviation of the laparoscopic group was 43 ± 15 years with a range of 15 to 82 years. The age range for the open cholecystectomy patients was identical, and the mean age was almost the same (44 ± 15 years). Women comprised the majority of both groups but they were significantly more predominant among the laparoscopic patients (83% vs. 66%, $P = 0.001$).

Indemnity Payments Accruing from Laparoscopic vs. Open Cholecystectomies

Fig. 1 shows the frequency distribution of NAIC scores for open and laparoscopic cholecystectomies.

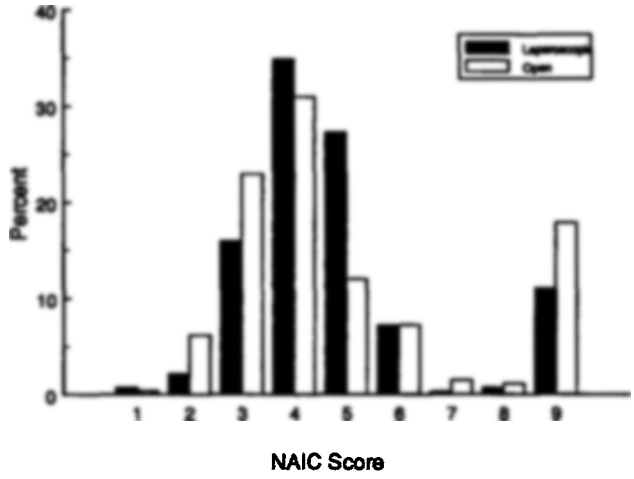

Fig. 1. Frequency distribution of National Association of Insurance Commissioners *(NAIC)* severity index code scores among laparoscopic and open cholecystectomy claimants.

Table II. NAIC scores and claims outcomes for laparoscopic and open cholecystectomies

Parameter	Laparoscopic	Open	P
No. of claims	306	261	–
NAIC score (mean ± SD excluding 9)	4.8 ± 1.8	4.0 ± 2.2	0.0003
Unresolved claims	84 (27%)	59 (23%)	
Closed without payment	107 (35%)	122 (47%)	0.017
Closed with payment	115 (38%)	80 (31%)	
Surviving claimants (mean ± SD)	$160 ± 154 × 10^3	$106 ± 122 × 10^3	0.01
Deceased claimants (mean ± SD)	$223 ± 171 × 10^3	$238 ± 291 × 10^3	0.88

Table III. Biliary tract injuries

Injury	No.	Cholangiogram (%)	Recognized (%)	NAIC score* (mean ± SD)	Deaths
Minor laceration and bile leak	100	41	23	4.1 ± 1.0	5
Clip encroachment or stricture	31	31	17	4.5 ± 1.0	2
Transection/resection	70	42	22	4.6 ± 1.0	3
TOTAL	201	40	22	4.3 ± 1.0	5%

*Excludes NAIC score of 9 (death).

The distributions were significantly different (P = 0.010) in the open group, driven primarily by the higher percentage of deaths. When the 32 deaths (11%) in the laparoscopic series and the 49 deaths (20%, P = 0.010) in the open series were excluded, the mean NAIC score for surviving laparoscopic claimants exceeded that for open cases (Table II). Claims of surviving laparoscopic litigants were more likely to be resolved with payment and less likely to be resolved without payment than those of open cholecystectomy survivors. Mean awards to surviving laparoscopic claimants were, on average, 1.5 times those paid to surviving open procedure claimants. Indemnity payments for death, on the other hand, were statistically equivalent for the two groups.

Injury Recognition and Outcome

Seventy-five percent of the injuries associated with laparoscopic cholecystectomy were not recognized before the patient left the operating room, but unrecognized injuries were not associated with a significantly greater likelihood of fatal outcome or with a significantly higher probability of indemnity payment to surviving claimants. A confounding factor was that some recognized injuries were not repaired or were repaired in a suboptimal manner; as a consequence, fully 95% of laparoscopic injuries resulted in a need for at least one additional surgical procedure.

Biliary Tract Injuries

There were 201 biliary tract injuries (Table III) accounting for almost two thirds of all alleged mishaps. Patients incurring biliary tract injuries were, on average, younger (mean age 40 ± 13 years) than those with other injuries (mean age 49 ± 16 years, P = 0.0001). Biliary injuries, in general, or resection/transection injuries, specifically, were not significantly more common in patients who had acute cholecystitis as the indication for their operations and were equally likely to occur whether or not cholangiography was performed. However, the latter observation cannot be interpreted in a meaningful way because the timing and quality of the cholangiograms were not stated.

Indemnity payments were significantly more likely for more serious biliary tract injuries—that is, minor lacerations and bile leaks involved payment in 34% of settled claims, clip encroachment and strictures required payment in 60%, and transections/resections involved payment in 78% (P = 0.001). Awards scaled upward from $124 ± 138 × 10^3 to $221 ± 179 × 10^3 for the least and most severe injuries, respectively (P = 0.048).

Vascular Injuries

Twenty-one vascular injuries occurred in 18 patients, as shown in Table IV. Eighteen (86%) of these were major vascular injuries, including one near-tran-

Table IV. Vascular injuries

Structure injured	Trocar	Recognized	Fatal	NAIC score* (mean ± SD)
Aorta	6	4	2	4.3 ± 1.0
Common iliac artery	9	6	2	4.5 ± 0.8
Inferior vena cava	2	1	2	—
Inferior epigastric artery	1	0	0	4
Mesenteric vessels	3	2	2	2
TOTAL	21	57%	24%	4.0 ± 1.2

*Excludes NAIC score of 9 (death).

Table V. Electrothermal and nonvascular trocar injuries

Structure injured	Trocar	Electrothermal	Recognized	Fatal	NAIC score* (mean ± SD)
Stomach	1	0	1	0	5
Hepatic duct	0	1	0	0	5
Common bile duct	1	4	0	0	4.2 ± 0.4
Duodenum	1	4	0	3	4.5 ± 0.7
Small bowel	15 − (*x*)†	(*x*)	2	3	4.7 ± 1.2
Colon	5†	(*x*)	2	1	3.8 ± 0.5
Urinary bladder	1	0	1	0	6
Remote skin	0	3	1	0	3.7 ± 0.6
TOTAL	24 − (*x*)	12 + (*x*)	20%	21%	4.4 ± 1.0

*Excludes NAIC score of 9 (death).
†Includes small but unknown number of electrothermal injuries (*x*).

section of the superior mesenteric vein. Introduction of the first port trocar appeared to be the cause in every instance. Two inferior vena cava injuries occurred in association with two of nine common iliac artery injuries; both patients died. Patients with recognized aortic and common iliac artery injuries all survived, with the exception of one case in which the inferior vena cava was also injured and, incidentally, adjacent colon and mesentery as well.

Electrothermal and Nonvascular Trocar Injuries

Thirty-six nonvascular injuries were caused in 34 patients by one or the other of these modalities (Table V). There were 26 gastrointestinal injuries, most of which were puncture wounds, except for the duodenum, which was usually thermally injured. All five duodenal lesions were not recognized during the original procedure, even though one duodenal injury, which also involved the common bile duct, was clearly stated to have been caused by a trocar. Three of four electrothermal duodenal injuries were fatal. Usually the other gastrointestinal lesions were easily assignable as puncture wounds caused by a trocar (or, rarely, a Veress needle) because they were clearly lacerations that leaked early, as opposed to late-presenting perforations; these lesions were in the ileum or sigmoid colon or they were injuries associated with major vascular puncture wounds. However, one colon perforation and several intestinal perforations at unspecified sites may have been stray current or laser beam injuries as indicated in Table V.

Most of the electrothermal injuries seemed to have been in the expected field of vision. With the exception of the hepatic duct injury, they were either not recognized or were apparently thought to be innocuous surface burns. Similarly, only one of three remote-skin electrothermal injuries was recognized, even though they were all in areas that were readily visible once the drapes had been removed; these injuries were situated on the face near the nose (recognized), on the chin, and on the external aspect of one arm.

Thirty-eight percent of 31 resolved claims result-

ing from electrothermal or trocar injuries (including vascular) involved indemnity payment, which was no different from the 37% payment incidence for other types of injuries. Mean payments associated with electrothermal and trocar injuries were $\$142 \pm 118 \times 10^3$ and $\$216 \pm 171 \times 10^3$, respectively, and were not statistically distinguishable from each other or from those associated with other injuries ($\$162 \pm 158 \times 10^3$).

Miscellaneous Injuries and Complications

These 47 injuries and complications had a collective mean NAIC score of 3.8 ± 1.1 (exclusive of scores of 9), which was not significantly different from the NAIC mean scores for the injuries described in Tables III and IV. These miscellaneous injuries and complications contributed a proportionate share (n = 7) of the 32 laparoscopic cholecystectomy deaths. Half of this group comprised 24 nonfatal and fatal myocardial infarctions, pulmonary infections, and other complications that bore only an indirect relationship to the performance of the procedure. The remainder included six instances of retained common bile duct stones, eight gallbladder hemorrhages, six cases of unretrieved stones within the peritoneal cavity, a nonfatal probable CO_2 venous embolism, and a port hernia. Eighty-one percent of these claims remained unresolved or were settled without indemnity payments. Indemnity payments were required in only 19% of closed miscellaneous group claims, as opposed to 41% for all other injuries ($P = 0.005$), and the mean payments were significantly less ($\$48 \pm 59 \times 10^3$ vs. $\$181 \pm 160 \times 10^3$; $P = 0.005$).

DISCUSSION

Laparoscopic cholecystectomy is a different operation from open cholecystectomy in several respects. The initial penetration of the peritoneum is usually not directly visualized, and dissection and hemostasis are largely energy based rather than relying on scissors and ligatures. Laparoscopically, caudal traction on the duodenum to open the triangle of Calot cannot be applied effectively. The target site for separating the gallbladder from the extrahepatic biliary system is the junction of the infundibulum of the gallbladder and the cystic duct rather than close to the merger of the cystic duct with the hepatic duct, as was traditionally practiced.[11,22] Magnification, superior illumination, and distention of the peritoneum provide better exposure and a better view of the field of interest albeit almost always two dimensional, than can usually be achieved in open celiotomies. Despite the better view and not needing to dissect the cystic-hepatic duct junction, energy-based dissection in a less splayed out triangle of Calot appears to pose more risk of injury to adjacent structures than was experienced in open cholecystectomies.[6]

The special skills and experience required for laparoscopic surgery have stimulated the formation of new surgical societies and have been a major factor in the revitalization of others. In a similar manner, recognition of the hazards inherent to port establishment and reliance on energy-based dissection, along with numerous published reports of biliary tract injuries, have stimulated the establishment of at least one special-interest group within the American Trial Lawyers Association devoted to laparoscopic procedures in general and to laparoscopic cholecystectomy in particular.[23] To examine the hypothesis that the plaintiff's bar, in selecting cases based on their potential for indemnity compensation, might emphasize certain injuries to the extent that they would be overrepresented in the present series, a surrogate for incidence was developed. The incidences of various injuries in the literature and the distribution of injuries in this report were normalized by dividing all figures by the number of biliary tract injuries characterizing each source, creating a relative incidence quotient for each injury (Table VI). The incidence used for the literature divisor was taken from the comprehensive review by Strasberg et al.[6] Literature citations for incidences of other injuries were not as well validated and usually depended on relatively few injuries[1-3,24,25] with the notable exception of the massive survey by Deziel et al.[5] Articles that dealt mainly with laparoscopic procedures other than laparoscopic cholecystectomy were excluded. The relative incidences in this series and in the literature were remarkably concordant, differing by a factor of two or less, with the exception of urinary bladder punctures, which involved only a single injury from each source. The plaintiff's bar appears to have brought injury allegations forward that mirror the relative incidence of laparoscopic cholecystectomy injuries as they are represented in the medical literature.

Nevertheless, the prosecution of alleged injuries on behalf of surviving claimants has been more successful following laparoscopic cholecystectomies than following open cholecystectomies. Claims of surviving laparoscopic litigants were significantly more likely to be settled with payment, and the payments were significantly larger than those made to surviving open-procedure claimants. The rationale for separating out indemnity payments to decedents was based on the premise that these awards were mainly influenced by lost potential earnings and the financial impact on the beneficiaries rather than reflecting the extent of negligence and injury inflicted on the patient. Since there was a significantly higher death rate among the open cholecystectomy patients, and payment amounts on behalf of dead claimants were high and equal in both

Table VI. Laparoscopic cholecystectomy injury relative incidence

Injury	Literature incidence per 1000*	Relative incidence normalized to biliary injury	
		Literature† (8.5/1000)[6]	Current series (201)
Biliary tract injuries	(6)	1.0	1.0
Minor biliary	3.3 (6)	0.39	0.50
Transection/resection	4.6 (3,5,6)	0.54	0.35
Major vascular	0.46 (5,14,24)	0.05	0.10
Stomach	0.06 (5)	0.007	0.005
Duodenum	0.16 (5,24)	0.02	0.03
Small bowel	0.35 (5,24)	0.04	0.08
Colon	0.44 (2,5,24)	0.05	0.03
Urinary bladder	0.01 (5)	0.001	0.005
Trocar (Veress) puncture	2.7 (22,24,25)	0.32	0.22
Electrothermal	0.43 (1,22)	0.05	0.06

*Numbers in parentheses are references cited.
†Calculated from average of referenced values.

groups, analyzing the data together would have obscured the significant differential in payments to surviving claimants.

In common with other reports, biliary tract injuries showed a biphasic distribution with substantial numbers of both minor injuries and major transections/resections.[2,5,6,8] It was assumed that recognizing a bile duct injury during the course of the procedure in which it occurred should have a mitigating influence on its litigious outcome, but such was clearly not the case. Closure without indemnity was no more likely for recognized injuries, and indemnity payments for injuries that were recognized and for those that were not were statistically identical. The major perturbing factor was failure to adequately treat the biliary tract injury, even if it was recognized, as has been reported by others.[9,10] Inability to detect a salubrious effect from prompt recognition was true, not just for biliary tract injuries but for the entire series. The likely confounder was again unsuccessful remedial actions because 25% of the injuries were recognized during the original procedure, yet 80% of these same patients had to undergo at least one additional operative procedure. We draw the same conclusion as Stewart and Way[9]: that a surgeon causing an injury must subordinate pride and fear of litigious consequences to putting the patient in a situation where he or she can receive the best care.

Most of the puncture wounds listed in Table V were actually caused by trocars, as judged by the magnitude of the hole or laceration, but some small bowel wounds were probably Veress needle punctures. The Veress needle was described in 1938[26] and it has enjoyed widespread use but is not fail-safe. The Hasson technique,[27] described in 1971, is generally advocated for establishment of the initial port in patients who have had previous abdominal surgery, but is not widely used for previously unentered abdomens, with the possible exception of pediatric laparoscopic procedures.[28] "Safety" trocars with spring-loaded sheaths, designed to work like the Veress needle, covering the sharp end of the trocar once it passes into the peritoneal cavity, are also not fail-safe. In fact, those with relatively thick plastic shields have been specifically implicated in trocar injuries because of the extra effort needed to push the shield through the transversalis fascia and peritoneum.[15]

Electrothermal injuries are particularly vicious because even if they are noticed, the depth of injury is difficult to assess and perforation of a viscus or bile duct typically occurs only after several days have elapsed.[18,29,30] In the case of electrothermal injuries of the biliary tract, perforation may not occur at all, and the result may instead be late stricture.[31] Electrothermal wounds produce coagulation necrosis and are easily distinguished from puncture wounds experimentally, but not necessarily after delayed clinical presentation.[32] As a consequence, a few of the puncture wounds listed in Table IV were actually electrothermal injuries. Similarly, we cannot be certain that all of the electrothermal injuries were due to electrosurgery because some may have been from the use of lasers, particularly early in the series. However, the majority of cases were accrued later, when it was apparent that lasers were more expensive, more hazardous, and no more effective than electrosurgery.[33,34]

Electrosurgery is widely used but poorly understood.[29,35] The field is burdened with imprecise terminology derived from its early grounded-circuit days. "Monopolar" and "bipolar" currents are both

actually bipolar, the differentiation being proximity or remoteness of the two poles and their size equivalency or disparity. "Cut" current will coagulate (desiccate), and "coag" current will cut.[36] Laparoscopic electrosurgical injuries can largely be avoided by judicious use.[28,36] The wattage should be set as low as it can be to still be effective. Most surgeons prefer "coag" current because it is more hemostatic, but "coag" current has an intermittent waveform, being off more than it is on, necessitating higher voltage than that needed for comparable wattage "cut" current.[36] Higher voltage is sometimes necessary, as an example, for noncontact fulgeration, but should generally be avoided because it increases the potential for arcing to nontarget tissue. Newer generators, manufactured by Valleylab Inc. (Boulder, Colo.) and Erbe (Tübingen, Germany, and Marietta, Ga.), constantly monitor impedance or voltage to maintain the preset wattage over a broad range of impedance, enabling lower wattage settings than needed for generators not having this instantaneous response.[37]

Most electrosurgical injuries are caused by isolated current paths that focus current density where it is not wanted, for example, cystic duct stump-to-common bile duct, or by inadvertent contact or sparking to adjacent structures. Some electrosurgical injuries, however, are due to stray current discharges from insulation defects or capacitive coupling, which can be eliminated by use of actively shielded monopolar electrodes (Electroscope, Boulder, Colo.), as recommended by the Emergency Care Research Institute (ECRI)[38] and others,[29,35] or by use of bipolar current.[30,36,39]

CONCLUSION

This series has shown that the spectrum of injuries coincident to laparoscopic cholecystectomy that has attracted the attention of personal injury lawyers is the same as that recorded in the medical literature, but is more likely to result in indemnity payments to surviving laparoscopic claimants than are injuries from open cholecystectomies. Should an injury occur in the course of laparoscopic surgery, its recognition has little impact, unless the injury is treated adequately. There are reasonable steps that can be taken to avoid many of these injuries. Risk-aversive measures should include use of proper technique when placing the first port, more liberal use of the Hasson technique, placement of all other ports under direct vision, elimination of intraoperative anatomic uncertainty, programmed inspection of the abdomen before the laparoscope is withdrawn, and sufficient knowledge of electrosurgical principles to ensure the safe use of this effective but potentially dangerous modality.

REFERENCES

1. The Southern Surgeons Club. A prospective analysis of 1518 laparoscopic cholecystectomies. N Engl J Med 1991;324: 1073-1078.
2. Go PMNYH, Schol F, Gouma DJ. Laparoscopic cholecystectomy in The Netherlands. Br J Surg 1993;80:1180-1183.
3. Russell JC, Walsh SJ, Mattie AS, Lynch JT. Bile duct injuries, 1989-1993: A statewide experience. Arch Surg 1996;131:382-388.
4. Fullarton GM, Bell G, the West of Scotland Laparoscopic Cholecystectomy Audit Group. Prospective audit of the introduction of laparoscopic cholecystectomy in the west of Scotland. Gut 1994;35:1121-1126.
5. Deziel DJ, Millikan KW, Economou SG, Doolas A, Ko ST, Airan MC. Complications of laparoscopic cholecystectomy: A national survey of 4,292 hospitals and an analysis of 77,604 cases. Am J Surg 1993;165:9-14.
6. Strasberg SM, Hertl M, Soper NJ. An analysis of the problem of biliary injury during laparoscopic cholecystectomy. J Am Coll Surg 1995;180:101-125.
7. Morgenstern L, McGrath MF, Carroll BJ, Paz-Partlow M, Berci G. Continuing hazards of the learning curve in laparoscopic cholecystectomy. Am Surg 1995;61:914-918.
8. Woods MS, Traverso LW, Kozarek RA, Tsao J, Rossi RL, Gough D, Donohue JH. Characteristics of biliary tract complications during laparoscopic cholecystectomy: A multiinstitutional study. Am J Surg 1994;167:27-34.
9. Stewart L, Way LW. Bile duct injuries during laparoscopic cholecystectomy: Factors that influence the results of treatment. Arch Surg 1995;130:1123-1129.
10. Schol FPG, Go PMNYH, Gouma DJ. Outcome of 49 repairs of bile duct injuries after laparoscopic cholecystectomy. World J Surg 1995;19:753-757.
11. Dubois F, Berthelot G, Levard H. Coelioscopic cholecystectomy: Experience with 2006 cases. World J Surg 1995;19: 748-752.
12. Jatzko GR, Lisborg PH, Pertl AM, Stettner HM. Multivariate comparison of complications after laparoscopic cholecystectomy and open cholecystectomy. Ann Surg 1995;221:381-386.
13. Steiner CA, Bass EB, Talamini MA, Pitt HA, Steinberg EP. Surgical rates and operative mortality for open and laparoscopic cholecystectomy in Maryland. N Engl J Med 1994; 330:403-408.
14. Apelgren KN, Scheeres DE. Aortic injury: A catastrophic complication of laparoscopic cholecystectomy. Surg Endosc 1994;8:689-691.
15. Saville LE, Woods MS. Laparoscopy and major retroperitoneal vascular injuries (MRVI). Surg Endosc 1995;9:1096-1100.
16. Lantz PE, Smith JD. Fatal carbon dioxide embolism complicating attempted laparoscopic cholecystectomy. J Forensic Sci 1994;39:1468-1480.
17. Cottin V, Delafosse B, Viale JP. Gas embolism during laparoscopy: A report of seven cases in patients with previous abdominal surgical history. Surg Endosc 1996;10:166-169.
18. Willson PD, McAnena OJ, Peters EE. A fatal complication of diathermy in laparoscopic surgery. Minim Invasive Ther 1994;3:19-20.
19. Physician Insurers Association of America Laparoscopic Procedure Study. Rockville, MD: Physician Insurers Association of America, 1994.
20. Sowka MP, ed. Malpractice claims: Final compilation. National Association of Insurance Commissioners, vol 2, No 2, 1980, pp 8, 52-65.

21. Hosmer DW, Lemeshow S. Applied Logistic Regression. New York: John Wiley & Sons, 1989.
22. Strasberg SM, Sanabria JR, Clavien PA. Complications of laparoscopic cholecystectomy. Can J Surg 1992;35:275-280.
23. Special Report on Laparoscopy Litigation Group. Laparoscopic Surgery Update 1995;3:121-123.
24. Litwin DEM, Girotti MJ, Poulin EC, Mamazza J, Nagy AG. Laparoscopic cholecystectomy: Trans-Canada experience with 2201 cases. Can J Surg 1992;35:291-296.
25. Larson GM, Vitale GC, Casey J, Evans JS, Gilliam G, Heuser L, McGee G, Rao M, Scherm MJ, Voyles CR. Multipractice analysis of laparoscopic cholecystectomy in 1,983 patients. Am J Surg 1992;163:221-226.
26. Veress J. Neues instrument zur Ausführung von Brust-oder Bauchpunktionen und Pneumothoraxbehandlung. Dtsch Med Woehenschr 1938;41:1480-1481.
27. Hasson HM. A modified instrument and method for laparoscopy. Am J Obstet Gynecol 1971;110:886-887.
28. Peters CA. Complications in pediatric urological laparoscopy: Results of a survey. J Urol 1996;155:1070-1073.
29. Voyles CR, Tucker RD. Education and engineering solutions for potential problems with laparoscopic monopolar electrosurgery. Am J Surg 1992;164:57-62.
30. Peterson HB, Ory HW, Greenspan JR, Tyler CW. Deaths associated with laparoscopic sterilization by unipolar electrocoagulating devices, 1978 and 1979. Am J Obstet Gynecol 1981;139:141-143.
31. Soper NJ, Flye MW, Brunt LM, Stockmann PT, Sicard GA, Picus D, Edmundowicz SA, Aliperti G. Diagnosis and management of biliary complications of laparoscopic cholecystectomy. Am J Surg 1993;165:663-669.
32. Levy BS, Soderstrom RM, Dail DH. Bowel injuries during laparoscopy: Gross anatomy and histology. J Reprod Med 1985;30:168-172.
33. Hunter JG. Exposure, dissection, and laser versus electrosurgery in laparoscopic cholecystectomy. Am J Surg 1993; 165:492-496.
34. Moossa AR, Easter DW, Van Sonnenberg E, Casola G, D'Agostino H. Laparoscopic injuries to the bile duct: A cause for concern. Ann Surg 1992;215:203-208.
35. Tucker RD. Laparoscopic electrosurgical injuries: Survey results and their implications. Surg Laparosc Endosc 1995; 5:311-317.
36. Nduka CC, Super PA, Monson JRT, Darzi AW. Cause and prevention of electrosurgical injuries in laparoscopy. J Am Coll Surg 1994;179:161-170.
37. Haag R, Cuschieri A. Recent advances in high-frequency electrosurgery: Development of automated systems. J R Coll Surg Edinb 1993;38:354-364.
38. Emergency Care Research Institute. Focus on Laparoscopy. Health Devices 1995;24:4-27.
39. Soderstrom RM. Electrical safety in laparoscopy. In Phillips JM, ed. Endoscopy in Gynecology. Downey, CA: American Association of Gynecologic Laparoscopists, 1978, pp 306-311.

Discussion

Dr. S. Strasberg (St. Louis, Mo.). I would like to emphasize a point that was not discussed because of time constraints. Of the 17 patients who had a major vascular injury, eight died. None of the patients who had a major vascular injury had the first port placed by the Hasson technique; in all of them the first port was placed by the Veress needle technique. Therefore this type of injury is completely preventable if the Hasson trocar technique is used, according to this report. I think there is a lesson here for all practitioners and educators. Only one biliary injury was described as "cautery associated." This seems somewhat low. Is it possible that there were other cautery-associated injuries that were not specifically recognized as such in the biliary injury group? Was there any litigation associated with a puncture or cautery injury to the bowel when the Hasson technique was used? Do you have any way of estimating the national incidence of this injury from your data?

Dr. J.G. Chandler. Some of the strictures of the biliary tract in the "clip encroachment or stricture" group were probably consequent to electrothermal injuries. I am sure we are underestimating the number of biliary electrothermal injuries. There were no injuries that were noted as being consequent to the Hasson technique, but I do not know how often the Hasson technique was actually used. The overall incidence of trocar puncture injuries is incredibly variable. There is one recent report citing only 20 cases ever being reported—it is obviously something people do not want to write about. I do not have any figures.

Dr. L. Way (San Francisco, Calif.). These are important epidemiologic data regarding the complications of laparoscopic cholecystectomy, and I suspect that analyses of medical negligence at this level will provide a useful overview of the case distribution, which will lead to a better understanding of the legal side of these events and will help indicate where to concentrate efforts at prevention. I would be cautious, however, in drawing conclusions concerning causation from data such as these. We have been studying causation of laparoscopic bile duct injuries for several years and have learned that the phenomenon is much more complex than originally suspected, and that the more information available, the more accurate the conclusion. The authors of this report did not have access to any of the following: (1) complete medical records, (2) initial and later operative notes, (3) pathologic findings, (4) autopsy reports, (5) videotapes of the operations, or (6) recollections of those who were directly involved (i.e., depositions). Under the circumstances, the mechanisms of injury would of necessity be speculative in most cases. So, although the recommendations are plausible and reasonable, they cannot be derived with confidence from the data. For example, in our review of bile duct injuries, we found little evidence of damage attributable to stray electrosurgical current. The injuries that involved electrocautery could virtually all be explained by direct contact of the injured tissue with an active electrode.

Laparoscopic vs. Open Intraoperative Ultrasound Examination of the Liver: A Controlled Study

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A prospective study was undertaken to determine whether the use of laparoscopy plus laparoscopic ultrasound examination can avoid unnecessary laparotomy, without missing potentially curable disease, in patients scheduled for curative liver surgery. Thirty-one consecutive patients who underwent surgery for planned curative liver surgery were prospectively evaluated by means of both laparoscopy plus laparoscopic ultrasound and laparotomy with intraoperative ultrasound. Laparoscopic ultrasound examination of the liver could not be performed in two patients, and in two other patients only partial examinations were possible because of dense adhesions. All patients underwent laparotomy with intraoperative ultrasound. A total of 50 malignant lesions were identified by laparoscopic ultrasound. All of these lesions were confirmed to be malignant at laparotomy with intraoperative ultrasound, that is, there were no false positive results. An additional four malignant lesions in four patients were not seen at laparoscopic ultrasound examination but were identified at laparotomy with intraoperative ultrasound (sensitivity 93%, specificity 100%, positive predictive value 100%, negative predictive value 85%). Based on the laparoscopic ultrasound findings, nontherapeutic laparotomy could have been avoided in 10% of our patients. Laparoscopy with laparoscopic ultrasound is a promising technology that may allow some patients to avoid a nontherapeutic laparotomy without significant risk of missing potentially curable disease. (J GASTROINTEST SURG 1997;1:146-151.)

Most patients in whom curative operations for hepatic tumors are attempted undergo preoperative imaging studies such as ultrasound, CT of the abdomen, CT portography, and occasionally angiography. This approach has a sensitivity of 85% for the detection of hepatic metastases.[1] The current gold standard for determining resectability of hepatic tumors involves laparotomy with visual and manual inspection plus intraoperative ultrasound (IOUS) examination of the liver. Using this approach, which has a sensitivity of 96%, additional lesions are sometimes found that may preclude curative surgery.[2-5] IOUS also provides additional information regarding relationships between tumors and vessels, which may aid in planning the surgical approach.

Laparoscopy with intraoperative ultrasound (LAP-US) is a relatively new technology whereby IOUS examination can be performed through 10 mm laparoscopic ports without the need for a laparotomy. Patients who undergo laparoscopy only can usually be discharged from the hospital either the same day or the day after surgery. Patients who undergo laparotomy for assessment of resectability, but no resection, generally remain in the hospital for approximately 7 days after surgery.[6] Laparoscopy has other well-recognized benefits such as less postoperative pain and more rapid return to normal activities. These benefits are particularly important with respect to the quality of life of patients with unresectable liver malignancies who generally survive only 6 to 12 months.[7] The current study was designed to determine whether the use of laparoscopy plus LAP-US can avoid unnecessary laparotomy, without missing potentially curable disease, in patients scheduled for curative liver surgery.

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METHODS

Thirty-one consecutive patients scheduled for potentially curative liver surgery (hepatic resection or cryosurgery) for malignant disease were studied from January 1994 to March 1996 (Table I). A predetermined protocol for management has been devised. Preoperative imaging studies used to select patients for surgery included abdominal ultrasound, abdominal CT, abdominal CT portography, and chest CT. Predetermined criteria for hepatic resection and cryosurgery are outlined in Table II. The study team in the operating room consisted of the surgical team, an interventional radiologist, and an ultrasound technician.

According to the protocol, the operative management for all patients included initial laparoscopy using a 30-degree laparoscope with dissection of adhesions and visual inspection of the abdomen. The falciform ligament was divided to allow the ultrasound probe to sweep over the entire surface of the liver. A LAP-US examination of the liver was then performed. The ultrasound probe used was the Bruel & Kjaer model 3535 (Bruel & Kjaer Instruments, Inc., Marlborough, Mass.), which has a convex array with color Doppler and multifrequency capability (5.0, 6.5, and 7.5 MHz). The tip of the probe can be flexed and hyperextended to allow effectual contact with the entire surface of the liver. In the first few patients, three 10 mm laparoscopic ports were used; however, we subsequently found that only two 10 mm laparoscopic ports were necessary (Fig. 1), allowing acquisition of sagittal and transverse images (Figs. 2 and 3). If suspicious lesions were identified that would preclude curative surgery, a laparoscopic biopsy, either direct or ultrasound guided, was performed. All patients without biopsy-proved disease precluding curative surgery proceeded to laparotomy with conventional IOUS examination and palpation of the liver. The probe used was the Bruel & Kjaer model 8544, which is a convex array sector probe with color Doppler and multifrequency capabilities. IOUS-guided biopsies were performed if suspicious lesions were identified that would preclude curative surgery or alter the planned procedure.

All lesions identified in the liver by means of LAP-US and IOUS were classified as either malignant or benign on the basis of ultrasonographic characteristics or biopsy results if available. According to ultrasound characteristics, most focal hepatic masses are nonspecific in appearance, with a wide differential diagnosis. There are, however, a few benign processes that have fairly characteristic appearances. A simple hepatic cyst is a well-defined hypoechoic structure without internal echoes, with a well-defined back wall and acoustic enhancement. Focal fatty infiltration is fairly classic predominantly in terms of its location. Typically this lesion is located just anterior to the portal vein and has a geographic appearance that is either hypoechoic within an otherwise hyperechoic fatty liver or hyperechoic within an otherwise normal liver.

Table I. Characteristics of liver tumors in 31 patients

Metastatic colorectal carcinoma	26
Hepatoma	3
Metastatic leiomyosarcoma	1
Cholangiocarcinoma	1

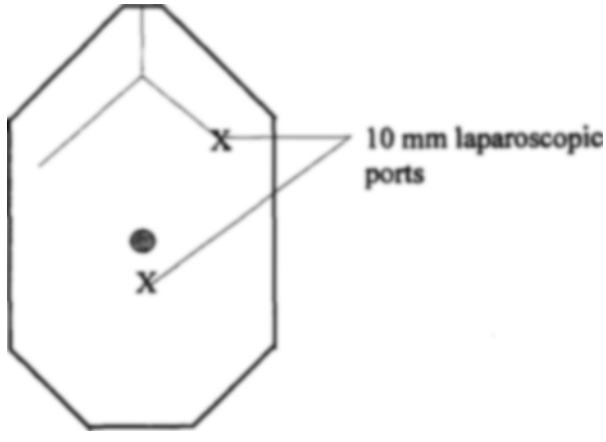

Fig. 1. Port position for LAP-US examination.

Table II. Predetermined criteria for cryosurgery and hepatic resection

Cryosurgery	Hepatic resection
No evidence of extrahepatic disease	No evidence of extrahepatic disease
Four or fewer lesions	Four or fewer lesions
No lesions >6 cm in diameter	All lesions within the confines of a segmental resection, formal lobectomy, or trisegmentectomy, with a minimum 1 cm resection margin
Lesions *not* within the confines of a segmental resection, formal lobectomy, or trisegmentectomy, with a minimum 1 cm resection margin	No comorbid conditions precluding planned resection
Presence of comorbid conditions precluding planned resection	

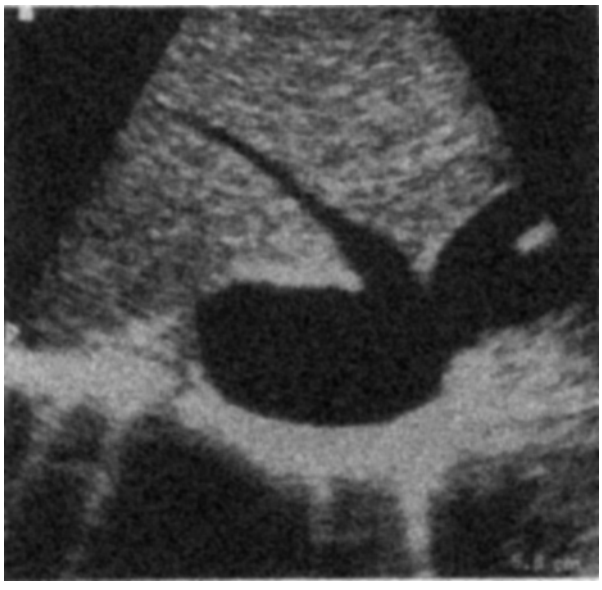

Fig. 2. LAP-US transverse image of liver at the confluence of the hepatic veins.

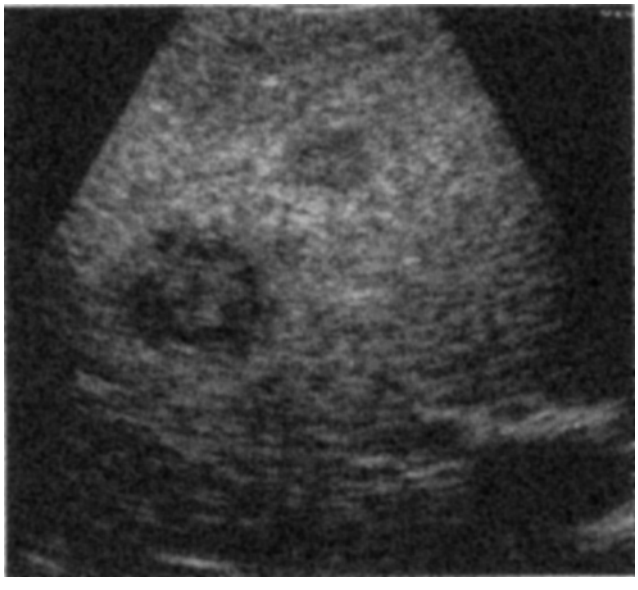

Fig. 3. LAP-US image of two colorectal liver metastases.

Hemangiomas are typically round hyperechoic solid-appearing masses. They are often peripherally located and their features may be enhanced through transmission.

For the purpose of data interpretation, the gold standard used was a combination of laparotomy plus IOUS and palpation of the liver with biopsy when appropriate. Pathologic examination of the resected specimen was not used as the gold standard for two reasons. First, five patients in the series had cryosurgery and only needle biopsies were performed. In addition, in those patients in whom hepatic resections were performed, only a portion of the liver was resected and thus the remaining liver was not available for pathologic examination. The resected specimens were examined by means of standard pathologic examination (1 cm sections) and the results correlated with laparotomy and IOUS findings.

Table III. Reasons for nonsurgical management

Patient No.	Description
2	Metastatic peripancreatic lymph node
13	Eight metastases*
24	Tumor invading porta hepatis*
26	Pelvic recurrence
27	Metastatic portal lymph node*
29	Pelvic recurrence

*Identified at LAP-US examination.

RESULTS

LAP-US was not possible in two patients because of dense adhesions. Two other patients underwent only partial LAP-US examinations because of adhesions. All patients underwent IOUS examinations and thus acted as their own control subjects for LAP-US. Results of standard pathologic examination of resected specimens correlated perfectly with IOUS findings and palpation, that is, no additional lesions were found and all resected tumors were malignant. Six patients were found to have disease that was not amenable to resection or cryosurgery (Table III). Three of these patients (10% of all patients) were identified by means of LAP-US, and thus nontherapeutic laparotomy could have been avoided. The patient with the metastatic peripancreatic lymph node was the second patient in our series. This is an area that is generally well seen with LAP-US and the lesion would likely have been detected as we have gained experience with this technique. There were two patients with recurrent tumors in the pelvis following previous anterior resection of the colon that were not detected by means of laparoscopy and LAP-US. Our protocol did not include extensive dissection of lower abdominal adhesions because of time constraints, and the pelvis was therefore not examined laparoscopically. The procedures performed in the

remaining 25 patients are outlined in Table IV. Using the criteria previously described for classifying lesions as either benign or malignant, 50 malignant lesions were identified at laparoscopy with LAP-US and confirmed at laparotomy with IOUS. Four lesions in four patients were identified at laparotomy with IOUS but not at laparoscopy with LAP-US, thus providing a sensitivity of 93% for LAP-US in this series (Table V). The details of the lesions that were missed by LAP-US are outlined in Table VI. Regarding the lesions not detected at LAP-US, there were no resulting alterations in the planned surgical approach in any of these patients.

Table IV. Procedures performed in remaining 25 patients

Right lobectomy	7
Left lobectomy	1
Left lateral segment resection	2
Trisegmentectomy	4
Segmental resection	6
Cryosurgery	5

Table V. Sensitivity and specificity of LAP-US for malignant lesions

		Laparotomy with IOUS +	Laparotomy with IOUS −
Laparoscopy with LAP-US	+	50	0
	−	4	23

Sensitivity = 93%; specificity = 100%; positive predictive value = 100%; negative predictive value = 85%.

DISCUSSION

Despite 5-year survival rates of 25% to 45% following hepatic resection for malignant disease, many patients still die of recurrent tumor. Many of these recurrences appear in the liver, suggesting that occult tumor was present at the time of the original resection.[8] These data have led to the development of more sensitive and specific imaging modalities to aid in the selection of patients for aggressive surgical therapy. Most hepatic surgeons use laparotomy with IOUS as the gold standard to select patients for curative surgery.[9]

Other groups have shown that laparoscopy plus LAP-US examination provides information that may preclude resection in up to 46% of patients.[10] We have chosen to compare laparoscopy plus LAP-US with the current gold standard, laparotomy with IOUS, to determine the sensitivity and specificity of laparoscopy plus LAP-US. Our data suggest that LAP-US examination is a sensitive test that would have allowed 10% of our patients to avoid a nontherapeutic laparotomy. It should be emphasized, however, that there were no false positive results in the series. Therefore LAP-US findings did not *inappropriately* suggest that plans for curative surgery be abandoned in any patients. It is also worth noting that this study represents the initial learning curve for this technique. Future studies are likely to show improved results as we become more familiar with the techniques and interpretations.

Our experience has identified some problems with the LAP-US examination. The procedure can be time consuming in patients with dense adhesions. As seen in Table VI, small superficial lesions that cannot be directly visualized with the laparoscope may be missed by LAP-US when the probe is placed immediately on top of the lesion. These lesions are usually palpable at laparotomy but often are not seen on IOUS. Thus, although LAP-US appears to be highly specific in identifying patients with incurable disease, occasional patients will still be found to have occult and incurable disease only at laparotomy. These problems may be overcome with more experience and better equipment in the future.

Table VI. Lesions missed by LAP-US

Patient No.	No. of lesions	Description
5	1	1 cm satellite adjacent to 5 cm right lobe lesion
6	1	Anterior subcapsular lesion appeared to be a cyst
13	1	Small superficial lesion posterior on right lobe
22	1	Small superficial lesion posterior on right lobe

CONCLUSION

We have shown that laparoscopy plus LAP-US is a practical and safe method for imaging the liver with a sensitivity approaching that of laparotomy with IOUS. According to our LAP-US findings, nontherapeutic laparotomy could have been avoided in 10% of our patients. More important, the positive predictive value of LAP-US is 100% and we can be confident that malignant tumors can be accurately differentiated from benign lesions by LAP-US.

REFERENCES

1. Nelson RC, Chezmar JL, Sugarbaker PH, Bernardino ME. Hepatic tumors: Comparison of CT during arterial portography, delayed CT, and MR imaging for preoperative evaluation. Radiology 1989;172:27-34.
2. Soyer P, Lacheheb D, Levesque M. False-positive CT portography: Correlation with pathologic findings. AJR 1993; 160:285-289.
3. Soyer P, Elias D, Zeitoun G, Roche A, Levesque M. Surgical treatment of hepatic metastases: Impact of intraoperative sonography. AJR 1993;160:511-514.
4. Machi J, Isomoto H, Kurohiji T, et al. Detection of unrecognized liver metastases from colorectal cancers by routine use of operative ultrasonography. Dis Colon Rectum 1986;29:405-409.
5. Soyer P, Levesque M, Elias D, Zeitoun G, Roche A. Detection of liver metastases from colorectal cancer: Comparison of intraoperative US and CT during arterial portography. Radiology 1992;183:541-544.
6. Conlon KC, Dougherty E, Klimstra DS, Coit DG, Turnbull ADM, Brennan MF. The value of minimal access surgery in the staging of patients with potentially resectable peripancreatic malignancy. Ann Surg 1996;223:134-140.
7. Bengtsson G, Carlsson G, Hafstrom L, Jonsson P. Natural history of patients with untreated liver metastases from colorectal cancer. Am J Surg 1981;141:586-589.
8. Fortner JG. Recurrence of colorectal cancer after hepatic resection. Am J Surg 1988;155:378-382.
9. Solomon MJ, Stephen MS, Gallinger S, White GH. Does intraoperative hepatic ultrasonography change surgical decision making during liver resection? Am J Surg 1994;168:307-310.
10. John TG, Greig JD, Crosbie JL, Miles WFA, Garden OJ. Superior staging of liver tumors with laparoscopy and laparoscopic ultrasound. Ann Surg 1996;220:711-719.

Discussion

Dr. W. Meyers (Worcester, Mass.). You had at least three persons interpreting the results of the ultrasound examinations. Is this your common practice?

Dr. V. Tandan. We have a very good relationship with our radiologist and that was our routine procedure for open intraoperative ultrasound before we began using laparoscopic ultrasound. We believe that the radiologists who perform ultrasound examinations every day probably carry out a more thorough examination of the liver than we can perform. There have been many times when we have begun ultrasound examination of the liver before the radiologists arrived. They have come in and found additional lesions that we did not see. We think that in today's day and age a multidisciplinary approach is probably the most desirable.

Dr. Meyers. Are these radiologists charging their professional fees?

Dr. Tandan. Yes. The health care system in Canada is publicly funded, and radiologists charge for their time and for biopsies when they perform them.

Dr. J. Milsom (Cleveland, Ohio). Was the radiologist who was with you in the operating room aware of the results of preoperative staging studies such as CT scanning? If such was the case, I believe this might have led to some bias on the part of the radiologist in the evaluation of the liver at the time of laparoscopy.

Dr. Tandan. You are absolutely correct. At the outset of the study, we had only one radiologist who was involved with the liver surgery and he did report both the preoperative and intraoperative ultrasound findings. We have since involved a second radiologist, and recently we have been randomly assigning radiologists to perform either the laparoscopic or the open intraoperative ultrasound examination in order to follow up on this study to obviate some of the bias that may have been introduced, as you pointed out.

Dr. B. Schirmer (Charlottesville, Va.). At what point will you feel comfortable doing without the radiologist? In other words, how many cases do you think will be necessary and how much experience will you need to acquire before you are able to carry on without a radiologist or do you plan to continue to use them indefinitely? At what point will you feel comfortable with your ultrasound findings such that you will not feel the need to perform open exploratory operations and unnecessary laparotomies can be avoided?

Dr. Tandan. We do not have any current plans to stop collaborating with our radiologist. We have started a second phase of the study in which we are randomly assigning radiologists and the surgical team so that we do not have the biases. I think after the completion of that study we will feel comfortable not recommending a laparotomy.

Dr. L. Way (San Francisco, Calif.). Have you tried to calculate the cost of acquiring all the information that is being obtained by following this strategy? It appears that at the present time you must perform both laparoscopic and open ultrasound examinations with the assistance of radiologists, which must be time consuming and costly. Of course, we are obliged to do everything reasonable that will benefit our patients, but we must also be as efficient as possible in this effort. Can you convince us that this is the most efficient way, or will it be possible to eliminate some of the extra persons involved in these cases?

Dr. Tandan. At the present time, this has all been done in

the context of a study protocol. It is important to remember that one of the fundamental principles of economic evaluations is that before such evaluations can be performed, therapy must be shown to be efficacious. Very often in clinical studies, the costs of controlling and monitoring the effectiveness of an intervention are significantly higher than they will be when the intervention is applied in practice. The objective of this study was to demonstrate whether laparoscopic ultrasound would be a suitable replacement for open intraoperative ultrasound; we do not plan on using both procedures once the laparoscopic procedure has been shown to be efficacious. Since we routinely perform laparoscopy prior to any major cancer surgery, the laparoscopic ultrasound examination should not add to the cost as long as it is replacing the open intraoperative ultrasound examination. If this were to occur, costs would likely be decreased, as some patients would not undergo nontherapeutic laparotomy and would probably require less time in the operating room and be discharged from the hospital earlier.

Dr. F. MacDonald (Scotland). You stated that you biopsy the lesions when appropriate. What proportion of lesions were biopsied? How often was the biopsy material unsatisfactory for histologic diagnosis? Was your sensitivity and specificity based on the total number of examinations performed or on the number of biopsy-proved lesions?

Dr. Tandan. We did not biopsy very many lesions. Approximately six lesions were biopsied and they were all confirmed to be malignant. Our sensitivity and specificity analysis is based on the total number of examinations performed.

Role of Computed Tomographic Arterial Portography and Intraoperative Ultrasound in the Evaluation of Patients for Resectability of Hepatic Lesions

Richard C. Karl, M.D., Junsung Choi, M.D., Timothy J. Yeatman, M.D., Robert A. Clark, M.D.

Computed tomographic arterial portography (CTAP) has been shown to be the most sensitive preoperative test for determining resectability of hepatic lesions but we have shown it to have low specificity. Intraoperative ultrasound (IOUS) evaluation of the liver has also been proposed as an accurate means of assessing resectability. We sought to compare the effectiveness of the two modalities. Fifty-six patients who had been deemed candidates for liver resection based on CTAP findings underwent systematic exploration, liver mobilization, and IOUS examination. Ultrasound findings were compared with results of CTAP. In 46 patients the IOUS findings were in complete agreement with those of CTAP. In 10 patients CTAP lesions could not be verified by IOUS and these patients did not undergo resection. Follow-up of these 10 patients revealed eight who did not have progression of malignancy at the CTAP-predicted site (CTAP false positive). Two patients did have progression at a CTAP-positive IOUS-negative site (IOUS false negative). Sensitivity for CTAP and IOUS was 100% and 96%, respectively. Specificity for IOUS was 100%. These findings demonstrate the high sensitivity of CTAP and the high sensitivity *and* specificity of IOUS. CTAP may "overcall" hepatic lesions but IOUS can correctly identify these false positives in most instances. Because CTAP is useful for determining which patients might benefit from surgical exploration, we conclude that the two modalities are complementary for the assessment of resectability of hepatic lesions. The false positive rate for CTAP implies that caution must be used when declining to operate on patients on the basis of this test. (J GASTROINTEST SURG 1997;1:152-158.)

Although surgical resection offers the best chance for surviving primary or secondary liver tumors, only a small percentage of patients will be deemed candidates for this treatment at the time they are first seen.[1-9] For this reason the selection of that subset of patients who will benefit from resection prior to surgical exploration becomes an important consideration in the management of these lethal diseases. Several preoperative imaging techniques have been evaluated for their efficacy in predicting resectability and detecting the presence of extrahepatic malignancy. These modalities have included computerized tomography (CT), delayed CT, arterial portography with CT (CTAP), magnetic resonance imaging, ultrasound, and hepatic artery perfusion scintigraphy. For the detection of intrahepatic lesions and depiction of their relationship to important intrahepatic vascular structures, CTAP has emerged in many studies as the most sensitive test, although it has a relatively high rate of false positive findings.[10-21]

Intraoperative ultrasound (IOUS) has been used with increasing frequency to assess the liver for the presence of malignant deposits and their relationship to segmental hepatic anatomy and vascular structures. Studies have been conducted to determine whether IOUS is more sensitive than CTAP and whether IOUS could be used in place of CTAP as the primary imaging modality for patients being considered for resection of hepatic malignancy.[7,22-24] Most investigators have concluded that IOUS is sensitive for the detection of liver malignancies and that it has a low rate of false positive results, but there is disagreement as to its role in the management of these patients.[7,22,23,25] Soyer et al.[23] reported no significant difference in the

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ability of CTAP and IOUS to detect hepatic malignancy and they concluded that IOUS is a useful technique that is "complementary" to CTAP.[7,22] In comparison, Fortunato et al.[26] found IOUS to be significantly more sensitive than CTAP for the detection of hepatic lesions and noted that IOUS was responsible for changes in proposed surgical management in one fourth of cases. We sought to compare IOUS and CTAP findings in 56 patients who underwent exploration for surgical management of hepatic malignancy.

MATERIAL AND METHODS

Of the patients referred to our institution for the management of hepatic malignancy, 106 underwent CTAP to assess their suitability for surgical exploration between April 1992 and December 1994. Of these, 56 patients (27 women [median age 67 years, range 29 to 81 years] and 29 men [median age 70 years, range 56 to 81 years]) underwent both exploration and IOUS evaluation. All patients with colorectal metastases were evaluated by means of CT of the chest and colonoscopy prior to CTAP.

Limited visceral angiography was performed to determine hepatic arterial anatomy and to position the tip of a 5 F RC-1 angiographic catheter in the superior mesenteric artery. CTAP was performed using a Somatom Plus-S or a Somatom Hi-Q or DRH scanner (Siemens Medical Systems, Inc., Iselin, N.J.). For the Plus-S system, 80 ml of nonionic contrast medium (iodine 320 mg/ml) was administered at a rate of 2 ml/sec by means of a power injector (Medrad, Inc., Pittsburgh, Pa.). Spiral scanning of the liver was performed 30 seconds after initiation of the bolus injection of contrast material; 8 mm sections were obtained and reconstructed at 5 mm increments. For the Hi-Q or DRH system, 80 ml of nonionic contrast material was given at a rate of 0.5 ml/sec, and dynamic scanning of the liver was performed after a delay of 20 seconds; 8 mm sections were obtained. Real-time IOUS examination was performed using a Toshiba diagnostic ultrasound scanner (model SSH-140A, Toshiba Medical Systems, Tokyo, Japan) with a 7.5 MHz linear array transducer (PVF-738F).

At the time of surgical exploration the ligaments suspending the liver were taken down to prepare the liver for bimanual palpation and ultrasonographic evaluation. A 7.5 MHz T-shaped transducer was placed directly on the surface of the liver after mobilization. Each segment of the liver was carefully scanned with reference to the preoperative CTAP (i.e., the IOUS was not blinded.) The operating surgeon performed the ultrasound examination. The lymph nodes along the hepatic artery and the common bile duct were routinely removed for frozen section diagnosis regardless of their appearance.

The results of CTAP and IOUS were compared. If a lesion was detected by one modality and not by the other, an attempt was made to biopsy the area in question. If the biopsy was negative for malignancy or if a suspicious area could not be confirmed, the area of liver in question was not resected and was followed by standard CT. Patients were followed with a baseline CT scan at 3 months and subsequent annual or biannual CT studies. Lesions seen on CTAP but not confirmed by IOUS were termed IOUS true negatives and CTAP false positives if subsequent follow-up failed to reveal any evidence of the development of malignancy in the disputed segment of liver. If subsequent follow-up demonstrated progression of disease at a site seen on CTAP but not on IOUS, these findings were classified as IOUS false negatives and CTAP true positives. There were no instances where IOUS revealed a lesion that had not been seen on CTAP. Because no patient was operated on without a positive CTAP, there were no CTAP true negatives in this study. Comparison of proportions for statistical significance was calculated using the chi-square statistic.

RESULTS

Fifty-six patients underwent preoperative CTAP, surgical exploration, and IOUS examination. The histologic types of malignancies in this group are shown in Table I. Four patients were known to have bilobar metastatic colorectal cancer and were operated on to place a hepatic arterial catheter for regional chemotherapy perfusion. Thus 52 patients were explored with intent to resect the portion(s) of liver found on CTAP to contain a lesion. Forty-seven patients underwent resection (resectability rate 90.4%). Twenty-eight patients underwent lobectomy, 13 had segmental resections, and six had metastasectomies. The reasons for abandoning liver resection in five patients are presented in Table II. In two instances lesions were determined to be unresectable by IOUS. In one case the tumor involved both the right and middle he-

Table I. Malignancies in 56 patients undergoing surgical exploration

Metastatic colorectal	(n = 49)
Hepatocellular	(n = 3)
Cholangiocarcinoma	(n = 2)
Pancreas	(n = 1)
Fallopian tube	(n = 1)

Table II. Reasons for abandoning liver resection in five patients

Extrahepatic disease (lymph node)	(n = 2)
Relationship to hepatic and portal veins	(n = 2)
Cirrhosis	(n = 1)

Table III. Reasons for test failure

CTAP false positives	IOUS false negatives
Median segment falciform ligament (n = 3)	Cyst (n = 1)
Gallbladder fossa (n = 2)	No lesion seen (n = 1)
Cysts (n = 2)	
Surface flow defect (n = 1)	

patic vein and in the other the malignancy abutted both the right and left branches of the portal vein. Thus the operative strategy was changed on the basis of the IOUS findings in 2 (3.8%) of 52 cases. In two instances lymph nodes along the hepatic artery were positive for malignancy despite their normal appearance. In one case of hepatocellular carcinoma, the patient's liver was much more cirrhotic than anticipated and plans for resection were abandoned. Thus the operative strategy was changed in 3 (5.8%) of 52 cases by intraoperative findings not related to imaging techniques. In 46 instances the results of preoperative CTAP were in complete agreement with those of IOUS, a situation we termed CTAP and IOUS true positive. In 10 patients there was a disparity between CTAP and IOUS findings. In each instance a lesion found on CTAP was not confirmed by IOUS. CTAP-detected lesions could not be seen on IOUS in nine cases and in one case the CTAP lesion was deemed a cyst. Nine of these patients underwent resection of a different portion of the liver and the area in dispute was not removed. One patient in this group did not undergo resection but was treated with regional perfusion. Four of 10 patients with discordant imaging findings have died. Three had shown no evidence of malignancy on postoperative CT scans in the disputed portion of the liver (follow-up at 13, 26, and 31 months) and were termed CTAP false positives and IOUS true negatives. One patient had progression of a lesion at a site determined to be positive by CTAP and a cyst by IOUS and died 17 months after the operation; this case was classified as a CTAP true positive and an IOUS false negative (Fig. 1). Six patients are still alive. One, who did not undergo resection, has had progression at a site considered positive by CTAP and negative by IOUS (follow-up 16 months). This was classified as a CTAP true positive and an IOUS false negative. Five patients are alive (follow-up 27.6 ± 1.3 months) with no evidence of disease. These cases were classified as IOUS true negatives and CTAP false positives. Thus each test was found to fail in a small number of cases. In eight patients CTAP false positives were due to flow artifacts in characteristic locations or to cysts (Table III and Fig. 2). In two cases of IOUS false negatives, the lesion was not seen in one and was thought to be a cyst in the other (see Table III and Fig. 1).

Table IV. Comparison of CTAP and IOUS values*

	CTAP	IOUS
Sensitivity	100%	96%
Specificity	N/A	100%
PPV	86%	100%
NPV	N/A†	80%

PPV = positive predictive value; NPV = negative predictive value.
*Differences between CTAP and IOUS were not significant.
†None of the patients classified as CTAP true negatives underwent surgery.

Table V. Patterns of recurrence after liver resection*

	No. (%)
Liver only	9 (36)
Liver plus distant site(s)	14* (56)
Extrahepatic only	2 (8)

*Twenty-five (53%) of 47 patients had a recurrence after resection.

Sensitivity, specificity, positive predictive value, and negative predictive value for each test are presented in Table IV. No significant differences were noted between the two tests. The odds ratio test value was 36.3, confirming a significant effect of IOUS on CTAP observations.

Of the 47 patients who underwent resection, 20 are still alive with no evidence of disease (28.9 ± 1.6 months) and two died of unrelated causes with no signs of recurrence (47%). Twenty-five patients (53%) have had a recurrence and 21 have died (17 ± 1.4 months). Four are still alive with disease (33.1 ± 7.2 months). The patterns of recurrence of malignancy are shown in Table V.

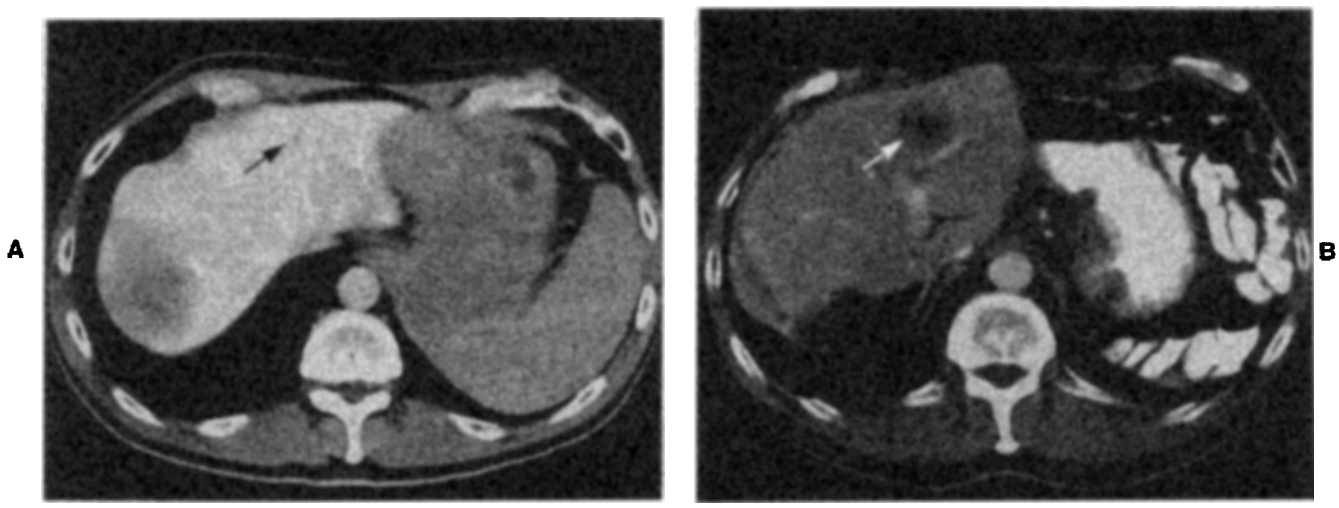

Fig. 1. A, Preoperative CTAP scan showing a lesion in the medial segment that was classified as a cyst at IOUS examination. **B,** Subsequent CT scan 14 months later showing progression at the disputed site (arrow).

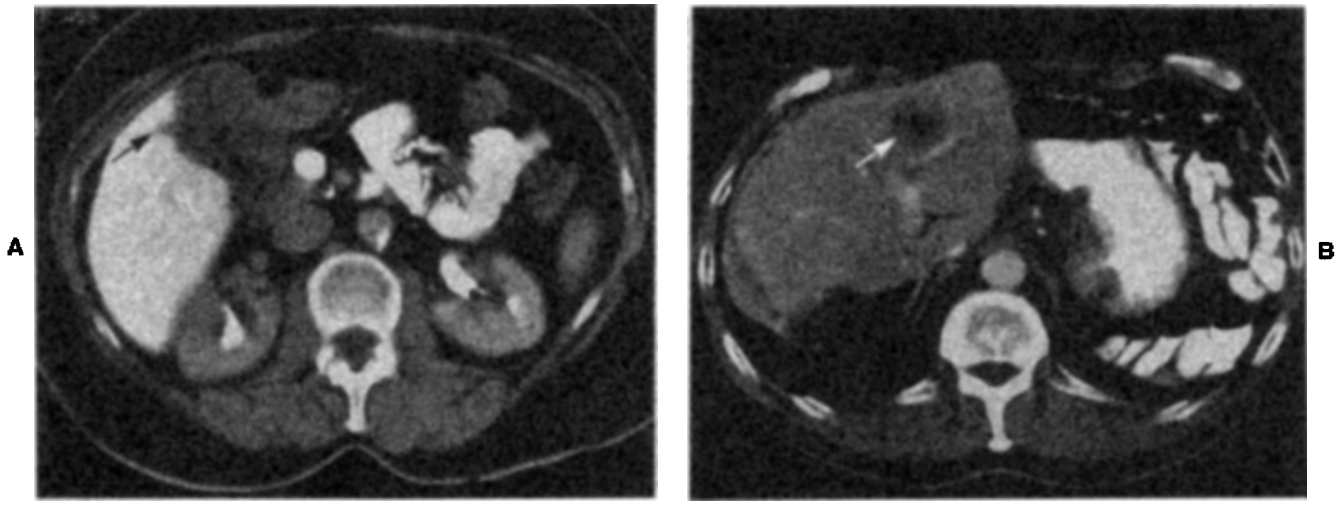

Fig. 2. Characteristic sites for CTAP false positive examinations. **A,** Gallbladder fossa. **B,** Medial segment.

DISCUSSION

Because a small but measurable fraction of patients with hepatic malignancy seem to be curable by liver resection, it is reasonable to attempt to identify that subset of patients who will be long-term survivors from the larger subset who will not benefit from an operation. The increasing safety of hepatic resection has prompted enthusiasm for resectional therapy, and over the past two decades some important principles of management of patients with these malignancies have emerged. Patients with a poor prognosis after liver resection include those with extrahepatic malignancy (even though it may be resectable), patients with satellite nodules of tumor or tumor thrombus in the hepatic vein, and patients in whom the margin of resection is less than 1 cm.[27-39] These observations

motivate the surgeon to be aware of the exact number of tumor nodules and their relationship to the hilar structures, the hepatic veins, and the vena cava.

The data reported herein are open to criticism because of the retrospective nature of the investigation, the highly select patient population, and the self-taught scanning technique employed by the surgeons performing that intraoperative test. Nonetheless, we noted some interesting factors that affect the management of patients with intrahepatic malignancy.

There are several methods that can be used to measure the specificity and sensitivity of imaging modalities. Imaging techniques can be compared with each other or with the findings in resected liver specimens. Another perhaps more clinically relevant method is to compare the results of imaging studies with the subsequent clinical fate of the remaining liver in patients undergoing resection. This method addresses the following question: if the imaging study declares a portion of liver to be free of disease, what is the likelihood that a malignancy will subsequently become evident in that portion? In-liver recurrence rates after resection of primary and secondary cancers range from 15% to 60% in many series, but the method of imaging the liver at the time of resection is often not made clear.[4,5,8,9,31] It is the biology of the hepatic malignancies, the accuracy of the imaging modalities, and the competency of the surgical procedure that affect long-term in-liver recurrence rates. In our patient population an overall 54% recurrence rate was observed. The liver-only recurrence rate was 36%. Because this study included patients with a variety of hepatic malignancies, it is not useful for drawing conclusions concerning the behavior of any specific type of liver cancer. Nonetheless, our liver-only recurrence rate is similar to that reported in other studies of patients with metastatic colorectal cancer.[31] Many factors affect the tumor recurrence rate after resection of a portion of the liver. Although a single cancer cell may lead to recurrence in the liver or metastasis elsewhere, it is not within the capability of current detection techniques to identify such a small focus of malignancy. It seems probable that the limitations of current imaging modalities are an important contributor to the high recurrence rate reported after resection of both primary and secondary hepatic malignancies. This study has sought to pose a question concerning current techniques for liver imaging in the management of primary and secondary liver tumors. Specifically, if CTAP and IOUS findings are in disagreement as to the presence of a malignant lesion in a particular portion of the liver that is not resected, what is the fate of that portion of the liver over time?

We have previously reported our experience with CT, CTAP, and delayed enhanced CT.[1] We agree with several other investigators who have found CTAP to be superior to other preoperative imaging studies for the detection of hepatic tumor deposits, although a relatively high rate of false positive findings has been noted.[1,11,12,40,41] These so-called pseudolesions are the result of arterial variations, catheter placement, and venous obstruction.[42] They are commonly located in segment 4, near the gallbladder fossa and along the falciform ligament[42,43] (see Fig. 2). The frequency of pseudolesions can be minimized by careful attention to catheter placement, adequate injection rates, and appropriate timing of CT scanning after injection.[42,43] Our CTAP false positive findings were mostly cases of "classic" pseudolesions, although cysts were mistaken for malignancies in two instances. We have previously reported the sensitivity and specificity for CTAP to be 97.1% and 33.3%, respectively.[1] In this study we found the sensitivity for CTAP to be 100% and for IOUS to be 96%. Although the specificity for CTAP cannot be calculated by definition in this study because there were no true negatives, specificity for IOUS was 100%. Thus IOUS correctly identified CTAP false positive examinations most of the time (80%).

Fortunato et al.[26] found IOUS to have a high sensitivity when compared with values for CTAP and CT (which were equivalent) and believed that the role of CTAP in preoperative evaluation of patients considered for liver resection should be re-evaluated. Furthermore, they claim that IOUS findings resulted in a change in management in one fourth of their cases. Our results do not agree. We have previously found CTAP to be much more sensitive than CT, and in this study we found sensitivity of IOUS to be equivalent to that of CTAP. The explanation for this disparity appears to be the relatively low sensitivity of CTAP in the Fortunato study. In addition, we found that IOUS was responsible for a change in our strategy only 3.8% of the time. The explanation for this difference may include the higher CTAP sensitivity in our study, the difference in referral patterns to different institutions, and differences in preoperative workup. These same factors may explain the relatively high resectability rate in our study. We conclude that CTAP with its spatial and contrast resolution is a good study to determine which patients warrant exploration with intent to resect liver lesions. Although the test may have a relatively high rate of false positive results, the well-known problem of pseudolesions can usually be resolved by IOUS. For this reason we agree with Soyer et al.[23] that the two techniques are com-

plementary. Patients with CTAP-detected lesions in locations characteristic of pseudolesions should not be denied exploration with intent to perform resection.

REFERENCES

1. Karl RC, Morse SS, Halpert RD, et al. Preoperative evaluation of patients for liver resection. Ann Surg 1993;217:226-232.
2. Sugarbaker PH. Surgical decision making for large bowel cancer metastatic to the liver. Radiology 1990;174:621-626.
3. Yamaguchi A, Ishida T, Nishimura G, et al. Detection by CT during arterial portography of colorectal cancer metastases to liver. Dis Colon Rectum 1991;34:37-40.
4. August DA, Ottow RT, Sugarbaker PH. Clinical perspectives on human colorectal cancer metastases. Cancer Metastasis Rev 1984;3:303-324.
5. Hughes KS, Simon RM, Songhorabodi S, et al. (members of the Hepatic Metastases Registry). Resection of the liver for colorectal carcinoma metastases: A multi-institutional study of indications for resection. Surgery 1987;103:278-288.
6. Sugarbaker PH, Kemeny MM. Management of metastatic liver cancer. In Tompkins RK, Balch CM, Cameron JL, et al., eds. Advances in Surgery, vol 22. Chicago: Year Book Medical Publishers, 1988, pp 1-55.
7. Foster JH. Survival after liver resection for secondary tumors. Am J Surg 1978;135:389-394.
8. Logans S, Meier S, Ramming K, et al. Hepatic resection of metastatic colorectal carcinoma. Arch Surg 1982;117:25-28.
9. Iwatsuki S, Esquivel CO, Gordon RD, et al. Liver resection of metastatic colorectal cancer. Surgery 1986;100:804-809.
10. Vogel SB, Drane WE, Ros PR, et al. Prediction of surgical resectability in patients with hepatic colorectal metastases. Ann Surg 1994;219:508-516.
11. Heiken JP, Weyman PJ, Lee JKT, et al. Detection of focal hepatic masses: Prospective evaluation with CT, delayed CT, CT during arterial portography, and MR imaging. Radiology 1989;171:47-51.
12. Nelson RC, Chezmar JL, Sugarbaker PH, et al. Hepatic tumors: Comparison of CT during arterial portography, delayed CT, and MR imaging for preoperative evaluation. Radiology 1989;172:27-34.
13. Small WC, Mehard WB, Langmo LS, et al. Preoperative determination of the resectability of hepatic tumors: Efficacy of CT during arterial portography. AJR 1993;161:319-322.
14. Soyer P, Levesque M, Elias D, et al. Detection of liver metastases from colorectal cancer: Comparison of intraoperative US and CT during arterial portography. Radiology 1992;183:541-544.
15. Merine D, Takayasu K, Wakao F. Detection of hepatocellular carcinoma: Comparison of CT during arterial portography with CT after intra-arterial injection of iodized oil. Radiology 1990;175:707-710.
16. Sitzmann JV, Coleman J, Pitt HA, et al. Preoperative assessment of malignant hepatic tumors. Am J Surg 1990;159:137-143.
17. Fortner JG, Silva JS, Golbey RB, et al. Multivariate analysis of a personal series of 247 consecutive patients with liver metastases from colorectal cancer. I. Treatment by hepatic resection. Ann Surg 1984;199:306-316.
18. Charnley RM, Binch C, Morris DL, et al. Visualization of detailed hepatic anatomy by intraoperative ultrasound. Br J Radiol 1988;61:769-770.
19. Castaing D, Edmond J, Kunstilinger F, et al. Utility of operative ultrasound in the surgical management of liver tumors. Ann Surg 1986;204:600-605.
20. Makuuchi M, Hasegawa H, Yamazaki S, et al. The use of operative ultrasound as an aid to liver resection in patients with hepatocellular carcinoma. World J Surg 1987;11:615-621.
21. Ravikumar TS, Kane R, Cady B, et al. Hepatic cryosurgery with intraoperative ultrasound monitoring for metastatic colon carcinoma. Arch Surg 1987;122:403-409.
22. Vogel SB, Drane WE, Ros PR, et al. Prediction of surgical resectability in patients with hepatic colorectal metastases. Ann Surg 1994;219:508-516.
23. Soyer P, Levesque M, Elias D, et al. Detection of liver metastases from colorectal cancer: Comparison of intraoperative US and CT during arterial portography. Radiology 1992;183:541-544.
24. Machi J. Preoperative evaluation of patients for liver resection: Appropriate CT imaging [letter; comment]. Ann Surg 1993;218:700-701.
25. Machi J, Isomoto H, Kurohiji T, et al. Accuracy of intraoperative ultrasonography in diagnosing liver metastasis from colorectal cancer: Evaluation with postoperative follow-up results. World J Surg 1991;15:551-557.
26. Fortunato L, Clair M, Hoffman J, et al. Is CT portography (CTAP) really useful in patients with liver tumors who undergo intraoperative ultrasonography (IOUS)? Am Surg 1995;61:560-565.
27. Surgical options in the treatment of hepatic metastasis from colorectal cancer. Curr Probl Surg 1995;337-421.
28. Scheele J, Stangl R, Altendorf-Hofmann A, et al. Indicators of prognosis after hepatic resection for colorectal secondaries. Surgery 1991;110:13-29.
29. Ekberg H, Tranberg KG, Andersson R, et al. Pattern of recurrence in liver resection for colorectal secondaries. World J Surg 1987;11:541-547.
30. Doci R, Gennari L, Bignami P, et al. One hundred patients with hepatic metastases from colorectal cancer treated by resection: Analysis of prognostic determinants. Br J Surg 1991;78:797-801.
31. Hughes KS, Simon R, Songhorabodi S, et al. Resection of the liver for colorectal carcinoma metastases: A multi-institutionaly study of patterns of recurrence. Surgery 1986;100:278-284.
32. Rosen CB, Nagorney DM, Taswell HF, et al. Perioperative blood transfusion and determinants of survival after liver resection for metastatic colorectal carcinoma. Ann Surg 1992; 216:493-505.
33. Sugioka A, Tsuzuki T, Kanai T. Post resection prognosis of patients with hepatocellular carcinoma. Surgery 1993;113: 612-618.
34. Hsu H, Wu T, Wu M, et al. Tumor invasiveness and prognosis in resected hepatocellular carcinoma, clinical and pathogenic implications. Cancer 1988;10:2095-2099.
35. Yamanaka N, Okamoto E, Toyosaka A, et al. Prognostic factors after hepatectomy for hepatocellular carcinomas. Cancer 1990;65:1104-1110.
36. Lai EC, Ng IO, You KT, et al. Hepatectomy for large hepatocellular carcinoma: The optimal resection margin. World J Surg 1991;15:141-145.
37. Chen M-F, Hwang T-L, Jeng BL, et al. Hepatic resection in 120 patients with hepatocellular carcinoma. Arch Surg 1989; 124:1025-1028.
38. Tang Z, Yu Y, Zhou X, et al. Surgery of small hepatocellular carcinoma. Cancer 1989;64:536-541.

39. Tsuzuki T, Sugioka A, Ueda M, et al. Hepatic resection for hepatocellular carcinoma. Surgery 1990;107:511-520.
40. Ferrucci JT. Liver tumor imaging, current concepts. Radiol Clin North Am 1994;32:39-54.
41. Freeny PC, Marks WM. Hepatic perfusion abnormalities during CT angiography. Radiology 1986;159:685-691.
42. del Pilar Fernandez M, Bernardino ME. Hepatic pseudolesion: Appearance of focal low attenuation in the medial segment of the left lobe at CT arterial portography. Radiology 1991;181:809-812.
43. Baum RA. CT during arterial portography: A review. Appl Radiol 1992;21:43-48.

Discussion

Dr. W. Meyers (Worchester, Mass.). If IOUS is so reliable, why not just eliminate CT?

Dr. R. Karl. I think this would result in operations being performed in a number of patients who would not benefit from surgical exploration.

Dr. Meyers. It could be done laparoscopically, couldn't it?

Dr. Karl. It could be done laparoscopically, although our experience with laparoscopic ultrasound is very limited.

Dr. Meyers. Regarding hepatomas, we have experienced a number of problems with flow artifacts. Have you not had similar problems?

Dr. Karl. There were only three cases of primary hepatocellular carcinoma in our group of patients. We have experienced the same flow defect artifacts that you describe. We still consider portography to be our mainstay for identifying patients who are suitable for surgical exploration.

Dr. B. Schirmer (Charlottesville, Va.). If CT portography is overly sensitive, what are your criteria for using it to exclude patients from surgical exploration?

Dr. Karl. We have previously presented data from patients whose CT findings were examined at the time they were initially seen; we compared these findings with results of CT portography and then operated on the patients. Forty-six percent of the time the patient's stage was changed between CT and CT portography. False positive rates for CT portography are approximately 10% to 13%. One may find bilobar disease, disease in areas that are not resectable, or unexpected lesions that are not seen on routine CT. Thus a large number of patients who would benefit from CT portography are identified. I think that in the future we will probably rely heavily on IOUS examinations performed through a laparoscope, which may replace CT portography. We currently still see a large percentage of patients, however, who are upstaged based on results of portography. When we observe lesions in these characteristic "false positive" locations (gallbladder fossa, medial segment, or flow defect on the surface) we are much more enthusiastic about operating on those patients than when two unsuspected lesions are identified at the confluence of the hepatic veins, for instance. So there are still a significant number of patients who benefit from portography.

Interleukin-10 Reduces Circulating Levels of Serum Cytokines in Experimental Pancreatitis

Anthony J. Rongione, M.D., Amy M. Kusske, M.D., Howard A. Reber, M.D., Stanley W. Ashley, M.D., David W. McFadden, M.D.

Over the past few years, evidence has accumulated that implicates proinflammatory cytokines as the mediators responsible for the escalation of acute pancreatitis into a multisystem disease. It has been shown that the degree of serum cytokine elevation, particularly the macrophage-derived cytokines interleukin-1, interleukin-6, and tumor necrosis factor-α, correlates with the severity and outcome of acute pancreatitis. Interleukin-10 is an anti-inflammatory cytokine that inhibits cytokine production from the macrophage. The aim of this study was to determine whether interleukin-10 would decrease both the severity of acute pancreatitis and the level of circulating proinflammatory cytokines. Ninety female mice were divided into three equal groups. Group 1 (controls) received intraperitoneal saline solution. Groups 2 and 3 received intraperitoneal cerulein (50 mg/kg/hr) for 7 hours. In addition, group 3 was given 1500 units of intraperitoneal interleukin-10, beginning 1 hour after the induction of acute pancreatitis and every 3 hours thereafter. Animals were killed at 3-hour intervals. Blood samples were obtained for serum amylase and cytokine determinations (interleukin-1β, interleukin-6, and tumor necrosis factor-α). Pancreata were dissected free and fixed in formalin for blinded histologic scoring. Interleukin-10 reduced the serum levels of interleukin-1β, interleukin-6, tumor necrosis factor-α, and amylase in comparison to untreated animals with pancreatitis ($P < 0.05$). Pancreatic edema, necrosis, and inflammatory cell infiltrate were also reduced in those animals given interleukin-10 ($P < 0.05$). Histologic score, serum cytokines, and amylase levels are elevated during acute pancreatitis. Interleukin-10 given therapeutically, that is, *after* the onset of acute pancreatitis, lessened the severity of disease, probably through inhibition of the macrophage. This was associated with a decrease in circulating cytokine levels. (J GASTROINTEST SURG 1997;1:159-166.)

Much evidence has accumulated over the past several years implicating proinflammatory cytokines as the mediators responsible for the progression of pancreatic inflammation into a multisystem disease. In fact, similarities between the sepsis syndrome and severe acute pancreatitis (e.g., adult respiratory distress syndrome, disseminated intravascular coagulation, circulatory failure, and multisystem organ failure) have led researchers toward the concept that common pathophysiologic mechanisms underlie both conditions. Inflammatory cytokines, released primarily by macrophages, are strong candidates as the mediators of these systemic sequelae. Indeed, as pancreatic destruction and enzyme release begin, the activated tissue macrophage may play a critical role in both the degree of local pancreatic inflammation and the systemic inflammatory response.

Grewal et al.[1] recently demonstrated that interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) levels were all elevated in the serum of animals with acute pancreatitis. Pretreatment with anti-TNF antibodies attenuated the expected increase in serum TNF-α, glucose, and amylase levels.[2] These findings were supported by Norman et al.,[3] who found that experimental pancreatitis was associated with the release of IL-1, IL-6, and TNF-α into the serum within an hour after the onset of the pancreatic insult. Their results demonstrated that the degree

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of serum cytokine elevation correlated with the severity and outcome of acute pancreatitis.[4] Furthermore, they showed that prophylactic and therapeutic treatment with an IL-1 receptor antagonist blunted the expected increase in these cytokines and was associated with decreased severity of pancreatitis.[5] Heath et al.[6] showed that serum levels of IL-6 from patients with acute pancreatitis correlated with the severity of the disease.

IL-10 is a recently characterized potent anti-inflammatory mediator that inhibits the production of other cytokines from activated macrophages.[7,8] The hypothesis to be tested in the present study was that IL-10 treatment would impair the release of proinflammatory mediators from activated macrophages, thus reducing the severity of pancreatitis. With a concern for the possible clinical relevance of the results, we studied the effects of IL-10 given *after* the initiation of the pancreatic insult, when the pancreatitis was already established.

MATERIAL AND METHODS

Materials

Cerulein was obtained from American Peptide Co., Inc. (Sunnyvale, Calif.). Recombinant murine IL-10 was a generous gift from the DNAX Research Institute (Palo Alto, Calif.). Aprotonin and amylase enzyme assay kits were obtained from Sigma Chemical Co. (St. Louis, Mo.). The Factor-Test-X Mouse TNF-α, the Intertest-1β-X Mouse Interleukin-1β, and the Intertest-6-X Mouse Interleukin-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Genzyme Diagnostics (Cambridge, Mass.).

Animal Model

All experiments were conducted with prior approval of the Animal Research Committee at Sepulveda VA Medical Center (Sepulveda, Calif.). Female Swiss-Webster mice, weighing 20 to 25 g, were purchased from Simonsen Laboratories (Gilroy, Calif.), fed standard laboratory chow, and allowed to acclimatize for a minimum of 1 week. Acute edematous pancreatitis was induced by hourly intraperitoneal injections of cerulein (50 mg/kg/hr) over a period of 7 hours, using a previously described technique.[9]

Animals were anesthetized at 3, 6, or 9 hours after the induction of pancreatitis by means of intraperitoneal injection of sodium pentobarbital (40 mg/kg). Laparotomies were performed and blood samples were immediately obtained from the inferior vena cava. The samples were collected in heparinized glass tubes containing aprotonin (0.009 trypsin inhibitory units/ml of blood). Serum was separated and stored at −70° C until assayed. Pancreata were immediately dissected free from their attachments and placed in formalin for histologic examination. A single investigator performed all dissections to limit technical differences. The severity of the pancreatitis was determined by measurement of serum amylase, serum TNF-α, IL-1β, and IL-6 levels, and blinded histologic grading was carried out by two investigators.

Experimental Design

Ninety female mice were divided into three equal groups. Group 1 (controls) received intraperitoneal saline solution. Groups 2 and 3 received hourly injections of intraperitoneal cerulein (50 mg/kg/hr) for 7 hours. In addition, group 3 was given 1500 units of intraperitoneal IL-10 diluted in 0.1% bovine serum albumin as a protein carrier, 1 hour following cerulein induction of acute pancreatitis and every 3 hours thereafter. Animals were killed at 3-hour intervals.

Assays

Serum amylase levels were measured at 37° C by enzymatic assay using a spectrophotometer according to the manufacturer's instructions. The rate of increase in absorbance at 405 nm is directly proportional to the pancreatic amylase activity. Serum TNF-α, IL-1β, and IL-6 levels were determined by means of ELISA according to the manufacturer's instructions. All serum samples were assayed in duplicate and averaged at the end of the experiment.

Histologic Grading

Pancreatic sections were stained with hematoxylin and eosin, and graded in a blinded manner on a scale of 0 to 4 to assess the degree of vacuolization, inflammation, necrosis, and edema.

Statistical Analysis

Differences between groups were evaluated by means of paired Student's *t*-test. Differences of $P < 0.05$ were considered significant. Results are expressed as mean ± standard error of the mean (SEM).

RESULTS

Serum Amylase

Following induction of pancreatitis, the serum levels of amylase in untreated animals increased compared to saline controls (group 2 vs. group 1; $P < 0.001$). IL-10 given after cerulein-induced pancreatitis significantly reduced serum amylase levels ($P < 0.001$ vs. group 2 given cerulein alone) (Fig. 1).

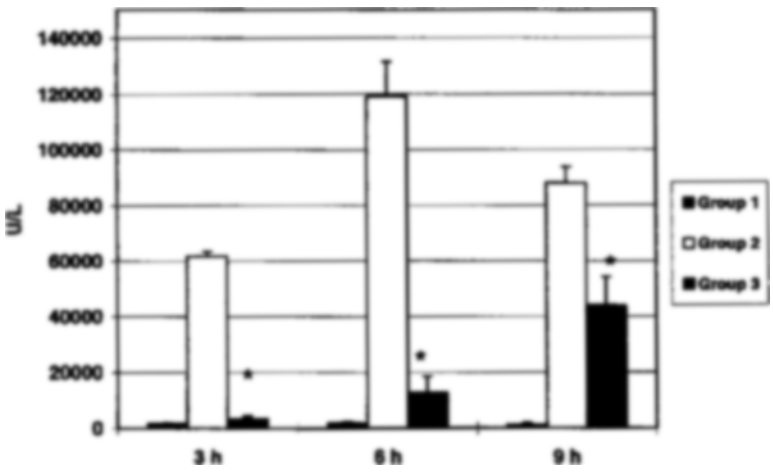

Fig. 1. Serum amylase profiles. Results are expressed as mean values ± SEM. Elevation of serum amylase is shown after the induction of acute pancreatitis. Following cerulein induction of acute pancreatitis, the serum amylase level in untreated animals increased in comparison to saline controls. Therapeutic treatment with IL-10 significantly reduced the increase in serum amylase levels at all time points evaluated ($P < 0.001$ vs. cerulein alone).

Table I. Histologic scoring of cerulein-induced pancreatitis in mice

Time after cerulein	Group 1 (saline controls)	Group 2 (cerulein alone)	Group 3 (cerulein + IL-10)
3 hours			
Edema	0	2.34 ± 0.19	0.83 ± 0.19*
Inflammation	0	1.81 ± 0.17	1.29 ± 0.12
Necrosis	0	1.65 ± 0.18	0.04 ± 0.05*
6 hours			
Edema	0	2.41 ± 0.15	1.50 ± 0.17*
Inflammation	0	2.23 ± 0.16	1.51 ± 0.14*
Necrosis	0	2.13 ± 0.14	0.61 ± 0.18*
9 hours			
Edema	0	2.84 ± 0.19	1.79 ± 0.18*
Inflammation	0	3.33 ± 0.20	1.75 ± 0.18*
Necrosis	0	3.51 ± 0.17	0.77 ± 0.17*

Data are expressed as mean ± SEM.
*$P < 0.001$.

Serum Cytokines

Serum cytokine profiles are given in Fig. 2. Following induction of severe necrotizing pancreatitis, serum IL-1β levels were elevated at 3 and 6 hours in the groups receiving cerulein alone compared to the saline control group ($P < 0.001$ vs. group 1). Therapeutic administration of IL-10 significantly attenuated this response ($P < 0.05$ vs. group 2). Elevations in serum TNF-α and IL-6 levels were observed at 6 and 9 hours in group 2 animals compared to the group 1 control mice ($P < 0.001$ vs. group 1). Again IL-10 significantly diminished this response ($P < 0.05$ vs. group 2).

Histologic Changes

Table I shows the histologic scoring for all groups of animals. Significant edema, vacuolization, necrosis, and inflammation were found in the group 2 untreated animals when compared to the saline control group ($P < 0.001$). Treatment with IL-10 in group 3 significantly decreased the degree of edema, vacuol-

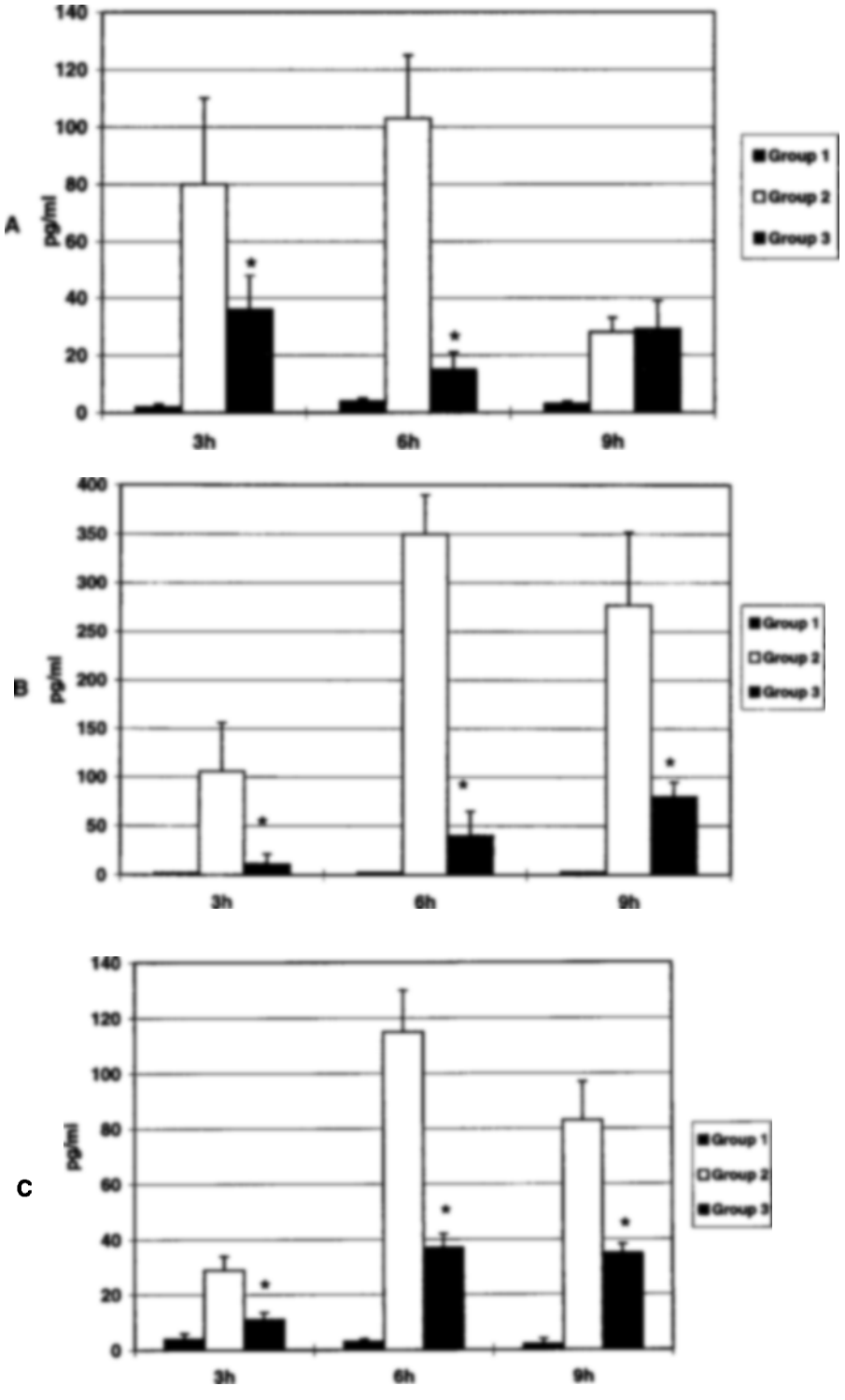

Fig. 2. Serum cytokine profiles. Results are expressed as mean values ± SEM. All cytokines measured were elevated after the induction of acute pancreatitis. IL-1β **(A)** showed the earliest increase at 3 hours and returned to baseline by 9 hours. IL-6 **(B)** and TNF-α **(C)** both peaked at 6 and 9 hours. The expected increase in IL-1β, IL-6, and TNF-α levels was significantly attenuated by therapeutic treatment with IL-10 ($P < 0.05$ vs. cerulein alone).

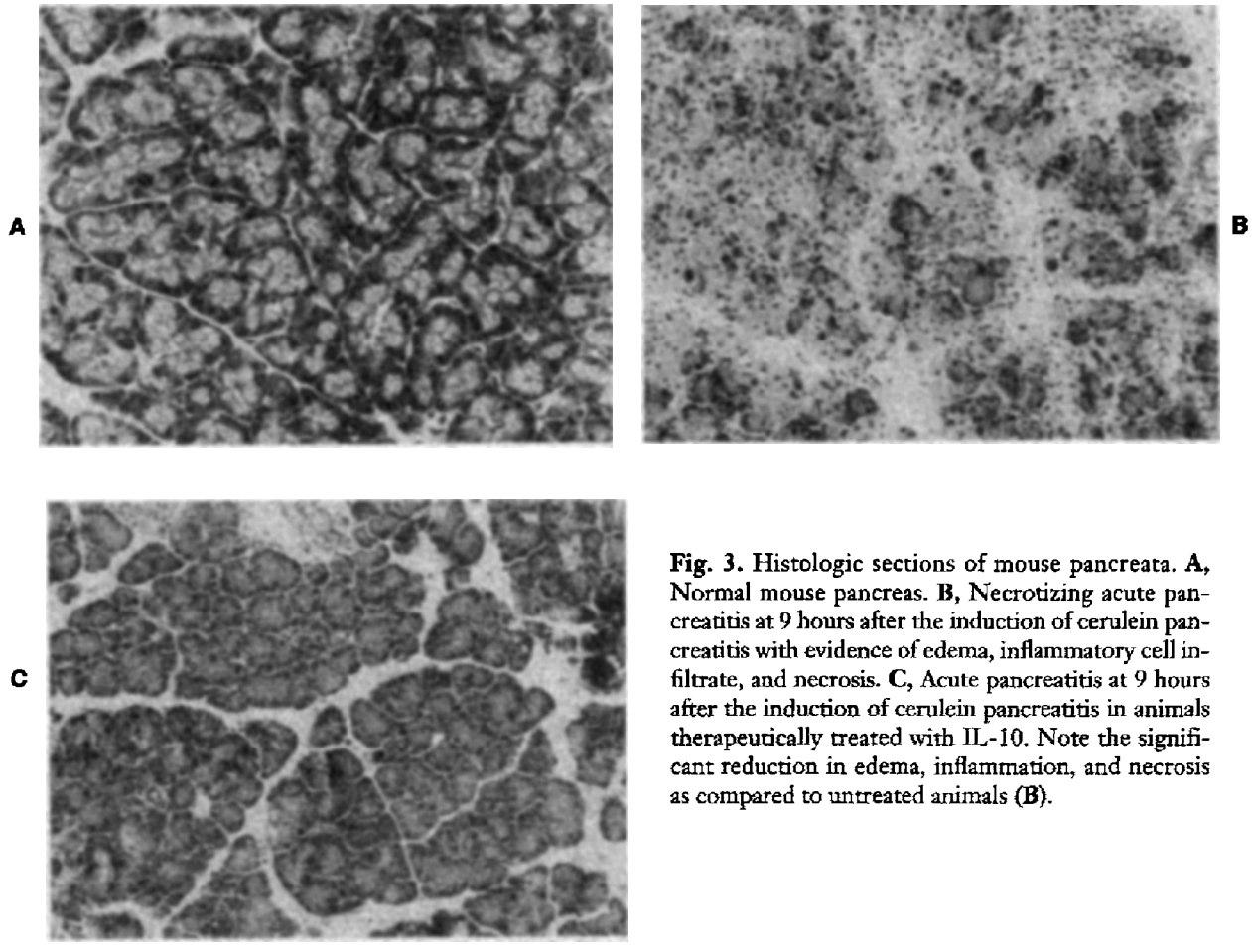

Fig. 3. Histologic sections of mouse pancreata. **A,** Normal mouse pancreas. **B,** Necrotizing acute pancreatitis at 9 hours after the induction of cerulein pancreatitis with evidence of edema, inflammatory cell infiltrate, and necrosis. **C,** Acute pancreatitis at 9 hours after the induction of cerulein pancreatitis in animals therapeutically treated with IL-10. Note the significant reduction in edema, inflammation, and necrosis as compared to untreated animals **(B)**.

ization, necrosis, and inflammation in comparison to group 2 untreated animals ($P < 0.001$). No statistical difference was observed between the group treated with IL-10 and the saline control group. Fig. 3 shows the characteristic histologic changes in the pancreata of groups 1, 2, and 3 at the 9-hour time point.

DISCUSSION

The mechanism by which pancreatic inflammation progresses into multiorgan system involvement remains largely speculative. However, recent developments suggest that both proinflammatory (IL-1β, IL-6, and TNF-α) and anti-inflammatory cytokines (IL-1ra and IL-10) may be critical factors in the pathogenesis of this sometimes fatal disease. For instance, IL-1β and TNF-α, released by the macrophage early in pancreatitis, regulate the inflammatory response by producing fever, metabolic abnormalities, and an immunologic response. These effects are mediated by the induction and secretion of other cytokines.[10] A major effect of IL-6, which is released later, is to induce acute-phase protein synthesis in the liver.[11]

The relative importance of TNF-α or IL-1 in the initiation and maintenance of the inflammatory response is unclear. Fong et al.[12] demonstrated that during lethal endotoxemia, TNF-α appears to stimulate the release of IL-1β and IL-6, which have both been implicated in the pathophysiology of multiorgan system failure in the sepsis syndrome. This is why anti-TNF therapy may be beneficial in the treatment of various inflammatory diseases. It could explain why Grewal et al.[2] and Hughes et al.[13,14] showed an improvement in the severity of experimental pancreatitis when anti-TNF-α antibodies were given. Others hypothesize that IL-1 may induce the production and secretion of TNF-α and IL-6, as well as itself.[15,16] This could explain why Norman et al.[4,5] found that IL-1 receptor antagonists ameliorated the severity of acute pancreatitis. These investigators also demonstrated that the production of TNF-α correlates with the pancreatic necrosis and the degree of severity of the pancreatitis.[17] TNF-α levels directly correlated with the infiltration of the activated macrophage, suggesting that the macrophage was the principal effector of pancreatic inflammation.

The foregoing data suggest that *both* TNF-α and

IL-1 play a significant role in the pathogenesis of acute pancreatitis. Therefore we theorized that more proximal cytokine antagonism at the level of the macrophage itself might be most effective. It should result in an almost complete attenuation of the expected increase in a number of the proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α). For this reason we chose to investigate the activity of IL-10 in this disease. IL-10 inhibits the secretion of proinflammatory cytokines by monocytes, macrophages, and T-helper cells.[18] It also has been shown to upregulate IL-1ra.[19] In vivo, IL-10 protected mice against lethal shock induced by staphylococcal enterotoxin B.[20] It also prevented death caused by experimental endotoxemia by reducing the release of TNF-α.[21]

Previous studies have shown that intraperitoneal IL-10 equilibrates rapidly into the circulation, with an apparent half-life of 2.5 ± 0.2 hours as measured by ELISA or 1.8 ± 0.3 hours as measured by bioassay.[22] For this reason, and based on the observation that multiple doses of IL-10 inhibit T-helper 1 cell–induced delayed-type hypersensitivity more effectively than a single dose, we administered the IL-10 every 3 hours.

In the present study we demonstrated an improvement in the histologic and biochemical markers of acute pancreatitis in mice when IL-10 was begun after the pancreatitis was already established. These effects also correlated well with the attenuation of serum cytokine levels. The fact that we were able to delay treatment with IL-10 suggests that there is a window of opportunity where administration of the drug precedes "full" activation of the macrophage. Alternatively, it may be that IL-10 is able to "turn off" cytokine production during an activated state. This idea is supported by de Waal-Malefyt et al.,[23] who showed that macrophages stimulated by lipopolysaccharide produced large amounts of IL-10 at a later time than IL-1, IL-6, and TNF-α. This suggests that IL-10 may be important to dampen the inflammatory reaction following antigenic challenge. Further studies should address IL-10 dosing at later time points, a clinically relevant scenario.

Van Laethem et al.[24] showed that *pretreatment* with IL-10 prevented pancreatic cellular necrosis in mice with cerulein-induced pancreatitis. It also lowered serum amylase, lipase, and pancreatic mRNA TNF-α levels. The IL-10 was given one-half hour before the first cerulein injection and again 4 hours later. In this nonlethal model of edematous pancreatitis, Van Laethem et al.[24] were unable to detect circulating TNF-α levels. They concluded that IL-10 had the ability to inhibit the synthesis of local proinflammatory mediators, as reflected by reduced TNF-α mRNA expression in pancreatic tissue. However, these investigators also found that IL-10 did not prevent the accumulation of inflammatory cells (neutrophils and/or macrophages) in the pancreas, even though cellular necrosis was inhibited. In contrast to their findings, we were able to prevent the typical histologic infiltrate during acute pancreatitis. This difference may be due to the variation in the dosing of IL-10, which was more frequent in our study, a difference in the purity or the potency of the material, or even a different response in the two animal species used. In any case IL-10 appears to have prevented neutrophil and/or macrophage recruitment in our model. In contrast to their observations, we found that the systemic levels of serum cytokines were elevated by the pancreatitis. This has also been found by Norman et al.[3] We can only speculate that this difference in findings may be a reflection of the particular ELISA employed for detection.

Our study does confirm, however, that TNF-α, IL-1β, and IL-6 are produced and released into the serum rapidly and that they correlate with the severity of the histologic and biochemical parameters. We interpret this to mean that the macrophage is an important mediator of the inflammatory response during acute pancreatitis. Several explanations for the benefits observed with IL-10 are possible. First, IL-10 is a potent inhibitor of the production of IL-1β, TNF-α, and IL-6, which are released from the activated macrophage. This could prevent or inhibit the accumulation of activated leukocytes in the inflamed pancreas. Second, IL-10 may impair the migration of inflammatory cells to the site of local inflammation. Indeed, Li et al.[22] showed that systemic administration of IL-10 could enhance migration of inflammatory cells into all tissues. If this had occurred in our study, fewer cells would have been available for migration to the site of pancreatic inflammation. Third, since macrophage recruitment precedes that of other effector cells during the early inflammatory response, IL-10, through its downregulation of the MHC II complex on the macrophage, may prevent recognition of the damaged pancreatic acinar cell (antigen) and thus inhibit the progression of the inflammatory response (neutrophils and T-helper cells).[25] Fourth, IL-10 is also known to upregulate anti-inflammatory cytokine expression, that is, IL-1ra, which has already been shown to lessen the severity of experimental pancreatitis.[4,5]

Previous work from our laboratory in other models of pancreatitis in rats and mice has led to conclusions similar to those drawn from the present study.[26-28] IL-10 appears to be an effective anti-inflammatory cytokine that ameliorates the biochemical and histologic para-

meters of acute pancreatitis ranging in severity from mild to lethal necrotizing disease. Since the effects of IL-10 are evident, even when it is administered after the onset of the pancreatic inflammation, investigation of its clinical utility now appears justified.

REFERENCES

1. Grewal HP, Malak K, El Din AM, et al. Induction of tumor necrosis factor in severe acute pancreatitis and its subsequent reduction after hepatic passage. Surgery 1994;115:213-221.
2. Grewal HP, El Din AM, Gaber L, et al. Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF-α polyclonal antibody. Am J Surg 1994; 167:214-219.
3. Norman JG, Franz MG, Riker A, et al. Rapid elevation of systemic cytokines during acute pancreatitis and their origination within the pancreas. Surg Forum 1994;45:148-150.
4. Norman J, Franz M, Fink G, et al. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 1995;221:625-634.
5. Norman J, Franz M, Messina J, et al. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery 1995;117:648-655.
6. Heath DL, Cruickshank DH, Gudgeon M, et al. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. Pancreas 1993;66:41-45.
7. Fiorentino DF, Zlotnik A, Mosmann TR, et al. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991;147:3815-3822.
8. de Waal Malefyt R, Yssel H, Roncarolo MG, et al. Interleukin-10. Curr Opin Immunol 1992;4:314-320.
9. Niederau C, Ferrell L, Grandell JH. Cerulein-induced acute necrotizing pancreatitis in mice: Protective effects of proglumide, benzotript and secretin. Gastroenterology 1985; 88:1192-1204.
10. Christou NV, Mannick JA, West MA, et al. Lymphocyte-macrophage interactions in the response to surgical infections. Arch Surg 1987;122:239-251.
11. Kishimoto T. The biology of interleukin-6. Blood 1989;74:1-10.
12. Fong Y, Tracey KJ, Moldawer LL. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1β and interleukin 6 appearance during lethal bacteremia. J Exp Med 1989; 170:1627-1633.
13. Hughes CB, Gaber LW, El Din ABM, et al. Inhibition of TNFα improves survival in an experimental model of acute pancreatitis. Am Surg 1996;62:8-13.
14. Hughes CB, Grewal HP, Gaber LW, et al. Anti-TNFα therapy improves survival and ameliorates the pathophysiologic sequelae of acute pancreatitis in the rat. Am J Surg 1996;171: 274-280.
15. Dinarello CA. Biology of interleukin-1. FASEB J 1988;2: 108-112.
16. DiGiovine FS, Duff GW. Interleukin-1: The first interleukin. Immunol Today 1990;11:13-20.
17. Norman JG, Fink GW, Franz MG. Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. Arch Surg 1995;130:966-970.
18. Fiorentino D, Zlotnik A, Mosmann T, et al. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991;147:3815-3822.
19. Howard M, O'Garra A, Ishida H, et al. Biological properties of interleukin-10. J Clin Immunol 1992;12:61-74.
20. Bean A, Freiberg R, Andrade S, et al. Interleukin 10 protects mice against staphylococcal enterotoxin B–induced lethal shock. Infect Immun 1993;61:4937-4939.
21. Howard M, Muchamuel T, Andrade S, et al. Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 1993; 177:1205-1208.
22. Li L, Elliott J, Mosmann T. IL-10 inhibits cytokine production, vascular leakage, and swelling during T-helper 1 cell-induced delayed-type hypersensitivity. J Immunol 1994; 153:3967-3978.
23. de Waal Malefyt R, Abrams J, Bennett B, et al. IL-10 inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; 174:1209-1220.
24. Van Laethem J, Marchant A, Delvaux A, et al. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. Gastroenterology 1995;108:1917-1922.
25. Moore KW, O'Garra A, de Waal Malefyt R, et al. Interleukin 10. Ann Rev Immunol 1993;11:165-190.
26. Rongione AJ, Kusske AM, Ashley SW, et al. Interleukin 10 reduces severity of acute pancreatitis in the rat [abst]. Pancreas 1995;11:446.
27. Kusske AM, Rongione AJ, Ashley SW, et al. Interleukin 10 (IL-10) prevents death in lethal necrotizing pancreatitis (AP) in mice [abst]. Surgery (accepted for publication).
28. Kusske AM, Rongione AJ, Lewis MPN, et al. Interleukin-10 (IL-10) reduces the severity of acute hemorrhagic pancreatitis in rats [abst]. Gastroenterol 1996;110:A407.

Discussion

Dr. S. Strasberg (St. Louis, Mo.). What is the longest time period from administration of the agents that cause the pancreatitis until you can get a response by administering IL-10?

Dr. A.J. Rongione. To date we have investigated early (1 hour) therapy in three different models of experimental pancreatitis ranging from mild to severe and found that IL-10 is effective in all of these models at this time point. Future studies need to be designed to determine how long after the induction of pancreatitis we can administer IL-10 and effectively treat the disease.

Dr. J. Norman (Tampa, Fla.). I have a problem with your last statement because I think it contradicts what you have shown. You stated that IL-10 decreases the severity of the pancreatitis and then decreases cytokine levels. I think it is the other way around. I would propose then that IL-10 decreased production of the cytokines, which therefore had the positive effect on the pancreatitis. Could you comment on the tissue levels, especially in the pancreas and lungs, of these cytokines.

Dr. Rongione. In response to your second question, after struggling with RNA and protein extraction from the

pancreas, we now have some data to suggest that mRNA and protein for TNF-α are decreased with IL-10 treatment. This is in a rat model of mild cerulein-induced pancreatitis. We have begun to study other cytokines, such as IL-1 and IL-6, now that our assays and protocols are working.

In response to the first question, I think what we have shown is that IL-10 inhibits the macrophage and decreases the release of cytokines. This inhibition of cytokines, as reflected by our lower serum cytokine levels, resulted in attenuation of the pancreatitis.

Dr. R. Printz (Chicago, Ill.). Your central hypothesis is that the macrophage plays a key role in the development of severe pancreatitis. Did you look at circulating levels of leukocytes in each of your groups? Did you examine the leukocytes that had infiltrated the pancreas in the different groups to determine whether there were any differences in these parameters?

Dr. Rongione. We have not studied circulating leukocyte counts, nor have we specifically marked the types of white cells that infiltrate the pancreas. I think there are data in the literature suggesting that the macrophage is the initial cell attracted to the pancreas during inflammation and that the severity of the pancreatitis correlates with the infiltration of the macrophage and subsequent recruitment of other cell types such as the neutrophil.

Dr. M. Sarr (Rochester, Minn.). In your summary you stated that IL-10 was given after the onset of pancreatitis. Everyone in the audience is questioning whether this is going to be something they can use. You really have not shown us that at 1 hour from the time you began your infusion of this agent, you had indeed induced pancreatitis. Do you have any data from the 1-hour time point?

Dr. Rongione. We do not have those data. We are basing our time points on data from other investigations that have clearly shown that by 1 hour there is established pancreatitis. Our goal is to prevent the systemic complications of pancreatitis. We believe that the pancreatitis is not what harms the patient; rather it is the systemic inflammatory response.

The focus in future studies will therefore be to attempt to attenuate the systemic inflammatory response in organs such as the liver and lungs. We may not, in fact, improve the pancreatitis with delayed dosing of IL-10 but, ideally, we would improve survival in severe, complicated pancreatitis.

Clinical Value of Diagnostic Laparoscopy With Laparoscopic Ultrasound in Patients With Cancer of the Esophagus or Cardia

H.J. Stein, M.D., S.J.M. Kraemer, M.D., H. Feussner, M.D., U. Fink, M.D., J.R. Siewert, M.D.

Accurate pretherapeutic tumor staging becomes increasingly important for the selection of therapy in patients with cancer of the upper gastrointestinal tract. We prospectively assessed the clinical value of diagnostic laparoscopy with laparoscopic ultrasound and peritoneal lavage in 127 consecutive patients with cancer of the esophagus or cardia but no evidence of hepatic metastases, peritoneal tumor dissemination, or other systemic tumor manifestations on standard staging techniques. There was no mortality or morbidity associated with diagnostic laparoscopy. Diagnostic laparoscopy with laparoscopic ultrasound showed relevant previously unknown findings, particularly in patients with locally advanced adenocarcinoma of the distal esophagus or cardia (hepatic metastases in 22% and peritoneal tumor spread or free tumor cells in the abdominal cavity in 25%), whereas the diagnostic gain was low in those with squamous cell esophageal cancer. The sensitivity and specificity of laparoscopic ultrasound in predicting positive celiac axis lymph nodes were 67% and 92%, respectively. These data indicate that diagnostic laparoscopy with laparoscopic ultrasound and peritoneal lavage is safe and frequently provides therapeutically relevant new information in patients with locally advanced adenocarcinoma of the distal esophagus or cardia, whereas the clinical value in patients with squamous cell esophageal cancer is limited. (J GASTROINTEST SURG 1997;1:167-173.)

With the availability of various therapeutic options in patients with cancer of the esophagus and cardia, the demand for more precision in pretherapeutic staging is growing, particularly if multimodality concepts are employed. Only accurate tumor staging allows an adequate selection of the ideal therapy for each patient and a correct assessment of the response to preoperative therapy.[1-4]

Although the evaluation of wall penetration in patients with carcinoma of the esophagus and cardia has become very accurate with the introduction of endoscopic ultrasonography, diagnostic gaps remain in the detection of small liver metastases, peritoneal tumor spread, and lymph node involvement.[1] It is, therefore, not uncommon that a scheduled curative resection must be abandoned after the intraoperative detection of a tumor that is more advanced than was appreciated on standard preoperative diagnostic tests.

In recent years diagnostic laparoscopy has been shown to be superior to CT and percutaneous ultrasound in the assessment of intra-abdominal tumor spread and resectability in patients with carcinoma of the hepatobiliary system, pancreas, or stomach.[5-9] The recent introduction of laparoscopic ultrasonography with a flexible ultrasound probe, the use of diagnostic lavage for the detection of free tumor cells in the abdominal cavity, and the opportunity to obtain biopsy specimens under direct visual or laparoscopic ultrasound control make diagnostic laparoscopy today even more appealing for staging of gastrointestinal malignancies.[10-13] In the present prospective study we attempted to determine whether diagnostic laparoscopy with laparoscopic ultrasonography and diagnostic lavage provides any diagnostic benefit in patients with cancer of the esophagus or cardia who had no evidence of systemic tumor spread on standard staging techniques.

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MATERIAL AND METHODS

Patient Population

In a prospective study diagnostic laparoscopy was performed between January 1994 and January 1996 in patients with carcinoma of the esophagus or cardia referred for surgical resection or multimodal therapy. Prior to diagnostic laparoscopy, the following tests were performed in all patients: endoscopy with endoluminal ultrasound and biopsy, standard chest x-ray examination, barium swallow, percutaneous ultrasonography of the abdomen, CT scan of the thorax and abdomen with intravenous and oral contrast, bone scan, and evaluation of hepatic, pulmonary, and renal function. Patients with hepatic metastases, other systemic tumor manifestations, or evidence of peritoneal tumor dissemination on these standard staging techniques were excluded from diagnostic laparoscopy. Patients who had previously undergone extensive upper gastrointestinal tract surgery and those whose overall physical condition was severely compromised were also excluded.

A total of 127 patients were included in the study. Demographic data relating to these patients are presented in Table I. There were 34 patients with squamous cell esophageal carcinoma located at or above the level of the tracheal bifurcation, 21 patients with squamous cell esophageal carcinoma located below the level of the tracheal bifurcation, 25 patients with adenocarcinoma of the distal esophagus (type I carcinoma of the gastroesophageal junction[14]), and 47 patients with adenocarcinoma located at or immediately below the anatomic cardia (type II and III carcinomas of the gastroesophageal junction[14]). Diagnostic laparoscopy with peritoneal lavage was performed in all patients. A complete laparoscopic ultrasound examination was possible in only 88 of 127 patients because of technical problems with the ultrasound probe during the initial phase of the study.

Technique of Diagnostic Laparoscopy With Laparoscopic Ultrasound and Peritoneal Lavage

Diagnostic laparoscopy was performed under general anesthesia according to a standardized protocol described in detail previously.[11,15] Briefly, three trocars (two 11 mm and one 5 mm) were used routinely; a fourth 5 mm trocar was placed when dissection of the lesser sac was difficult or when dense adhesions were present. The first trocar (11 mm) was placed in the midline above the umbilicus and was used for insertion of the laparoscope. A 30-degree laparoscope was employed in all instances. All additional trocars were placed under direct vision to the left and right of the first trocar and were used to insert a liver retractor and instruments for manipulation and dissection.

Prior to any manipulations, the entire abdomen was inspected and a diagnostic lavage of the upper abdomen was performed with 200 ml of physiologic saline solution. The hiatus, the lesser curvature, and the hepatoduodenal ligament were carefully inspected after elevation of the left lobe of the liver. In all patients the lesser sac was accessed through the gastrocolic or gastrohepatic ligament and evaluated for tumor manifestations. Laparoscopic ultrasonography was then performed with a flexible 7.5 mHz ultrasound probe inserted through the second 11 mm trocar. A screen divider allowed direct visual control of the position of the ultrasound probe during the ultrasound examination (Fig. 1). The entire liver, the hepatoduodenal ligament, the lesser curvature, and the celiac axis region were scanned for metastases or enlarged lymph nodes. All suspicious lesions were biopsied with the exception of the primary tumor and enlarged lymph nodes. Liver metastases were biopsied under direct vision or under laparoscopic ultrasound control using the screen divider.

At the end of the procedure, a second lavage with 200 ml of physiologic saline solution was performed to assess tumor cell spread that may have occurred

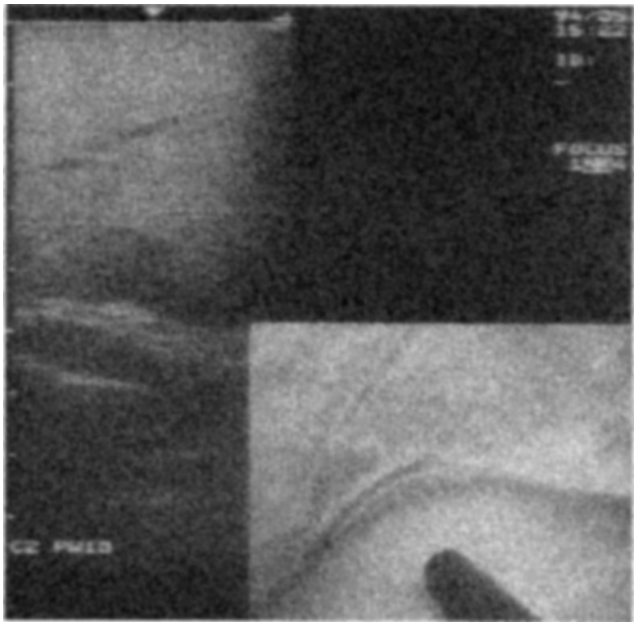

Fig. 1. Example of how a screen divider allows viewing of laparoscopic and ultrasound images on a single monitor. The laparoscopic image shows the position of the flexible ultrasound probe, the ultrasound image can be viewed simultaneously.

during the manipulations. The abdominal cavity was then rinsed with a cytotoxic solution of 0.5% taurolidine. The entire procedure was videotaped and all findings were documented on a standardized form.

Data Analysis

The prevalence of previously unknown hepatic metastases, peritoneal tumor dissemination, free tumor cells in the lavage, and liver cirrhosis was calculated for the entire patient population and all subgroups. The sensitivity and specificity of laparoscopic ultrasound, percutaneous ultrasound, and CT in the diagnosis of celiac axis lymph node metastasis were calculated by standard decision matrix using the histopathologic assessment of the specimen in patients undergoing primary resection as the "gold standard." Surgeons performing the diagnostic laparoscopy were blinded to the results of the other staging modalities.

RESULTS

There was no mortality or morbidity associated with diagnostic laparoscopy. As documented by the second abdominal lavage performed at the end of all manipulations, diagnostic laparoscopy did not result in any spread or mobilization of tumor cells. We did not observe any implantation metastases at the port sites in the present study.

Diagnostic laparoscopy and laparoscopic ultrasonography yielded 44 relevant new findings in 31 (24.4%) of 127 patients (Table II). None of these findings had been appreciated on standard preoperative staging modalities. All of the patients with newly detected liver metastases or frank peritoneal tumor spread were excluded from curative resection or multimodal protocols and palliative therapy was initiated. A total of 58 patients underwent primary resection, whereas 38 were included in neoadjuvant treatment protocols. Palliative therapy was instituted in the remainder.

Table III presents the relevant findings in diagnos-

Table I. Demographic data of the study population

	No. of patients	Male:female ratio	Mean age (yr)
Squamous cell esophageal cancer located at or above the tracheal bifurcation	34	29:5	52
Esophageal cancer located below the tracheal bifurcation			
Squamous cell carcinoma	21	17:4	54
Adenocarcinoma (type I)	25	22:3	62
Adenocarcinoma of the gastroesophageal junction (types II and III)	47	28:19	60
TOTAL	127	96:31	56

Table II. Relevant findings first detected by diagnostic laparoscopy with laparoscopic ultrasound and peritoneal lavage in 127 consecutive patients with carcinoma of the esophagus or gastroesophageal junction

	No. of patients	% of population
Liver metastasis	11	8.7
Liver metastasis and liver cirrhosis	1	0.8
Liver metastasis and peritoneal carcinosis and free tumor cells	4	3.1
Peritoneal carcinosis and free tumor cells	4	3.1
Free tumor cells	4	3.1
Liver cirrhosis	7	5.5
Normal laparoscopic findings	96	75.6

Table III. Relevant findings first detected on diagnostic laparoscopy according to location of the primary tumor

	Hepatic metastases	Peritoneal tumor spread	Free tumor cells on lavage	Liver cirrhosis
Squamous cell esophageal cancer located at or above the tracheal bifurcation	1/34 (2.9%)	0/34 (0.0%)	0/34 (0.0%)	5/34 (14.7%)
Esophageal cancer located below the tracheal bifurcation				
Squamous cell carcinoma	2/21 (9.5%)	0/21 (0.0%)	0/21 (0.0%)	2/21 (9.5%)
Adenocarcinoma (type I)	5/25 (20.0%)	3/25 (12.0%)	4/25 (16.0%)	0/25 (0.0%)
Adenocarcinoma of the gastroesophageal junction (types II and III)	8/47 (17.0%)	5/47 (10.6%)	8/47 (17.0%)	1/47 (2.1%)
TOTAL	16/127 (12.5%)	8/127 (6.3%)	12/127 (9.6%)	8/127 (6.3%)

Table IV. Relevant findings first detected on diagnostic laparoscopy according to histologic type and endosonographic T-stage of the primary tumor

	Hepatic metastases	Peritoneal tumor spread	Free tumor cells on lavage	Liver cirrhosis
Squamous cell esophageal cancer				
T1/T2 tumors	0/19 (0.0%)	0/19 (0.0%)	0/19 (0.0%)	2/19 (10.5%)
T3/T4 tumors	3/36 (8.3%)	0/36 (0.0%)	0/36 (0.0%)	5/36 (13.9%)
Adenocarcinoma of the distal esophagus (type I)				
T1/T2 tumors	1/9 (11.1%)	0/9 (0.0%)	0/9 (0.0%)	0/9 (0.0%)
T3/T4 tumors	4/16 (25.0%)	3/16 (18.6%)	4/16 (25.0%)	0/16 (0.0%)
Adenocarcinoma of the gastroesophageal junction (types II and III)				
T1/T2 tumors	1/13 (7.7%)	0/13 (0.0%)	1/13 (7.7%)	1/13 (7.7%)
T3/T4 tumors	7/34 (20.6%)	5/34 (14.7%)	7/34 (20.6%)	0/34 (0.0%)
TOTAL	16/127 (12.5%)	8/127 (6.3%)	12/127 (9.6%)	8/127 (6.3%)

tic laparoscopy classified according to histologic tumor type and location of the primary tumor. Previously unknown hepatic metastasis or peritoneal tumor spread was frequently observed in patients with adenocarcinoma of the distal esophagus (type I tumors) or gastroesophageal junction (type II and III tumors). In patients with squamous cell esophageal carcinoma, previously unknown liver metastases were uncommon, whereas liver cirrhosis was first detected by diagnostic laparoscopy and confirmed by laparoscopic biopsy in up to 15% of these patients. These differences were even more pronounced when patients with locoregional tumors (T1 or T2 tumors on endoscopic ultrasonography) were assessed separately from those with locally advanced disease (T3 or T4 tumors on endoscopic ultrasonography). Diagnostic laparoscopy with laparoscopic ultrasound and peritoneal lavage showed previously unknown hepatic metastases or peritoneal tumor dissemination in up to 25% of patients with locally advanced adenocarcinoma of the distal esophagus or gastroesophageal junction, whereas relevant new findings on tumor extension were uncommon in patients with squamous cell esophageal carcinoma (Table IV).

The sensitivity and specificity of laparoscopic ultrasound, percutaneous ultrasound and CT in predicting positive celiac axis lymph nodes are shown in Table V. Although laparoscopic biopsy of suspicious lymph nodes was omitted in the present study, the sensitivity of laparoscopic ultrasound in the diagnosis of positive celiac axis nodes was markedly higher as compared to percutaneous ultrasound or CT.

Table V. Sensitivity and specificity of laparoscopic ultrasound, percutaneous ultrasound, and CT in the assessment of celiac axis lymph nodes

	Sensitivity ("true positive")	Specificity ("true negative")
Laparoscopic ultrasound	67%	92%
Percutaneous ultrasound	35%	78%
CT	47%	82%

DISCUSSION

The current staging workup of patients with carcinoma of the esophagus and gastroesophageal junction is aimed at assessing the depth of wall penetration, lymphatic spread, and systemic metastases.[1] Although the staging of wall penetration can today be performed satisfactorily by endoscopic ultrasonography, there remains a diagnostic gap as far as intra-abdominal tumor dissemination and liver metastases are concerned.[16] Diagnostic laparoscopy has the potential to close this gap. Initial retrospective studies in patients with carcinoma of the esophagus and cardia have uniformly demonstrated the superiority of diagnostic laparoscopy over all other staging modalities in the detection of liver metastases, intra-abdominal lymph node metastases, and peritoneal tumor spread.[17-20] Recent advances have made diagnostic laparoscopy an even more complete staging tool for gastrointestinal malignancies. These include laparoscopic ultrasonography with a flexible ultrasound probe and diagnostic lavage to search for free tumor cells in the abdominal cavity.[11,21] The present study shows that with the use of these new techniques, diagnostic laparoscopy provides relevant additional information in up to 25% of patients.

Hepatic metastases in patients with carcinoma of the esophagus or cardia are associated with a dismal prognosis. Exact knowledge of their presence or absence is, therefore, essential before any extensive therapeutic approaches can be considered. Current modes for detecting liver metastases include percutaneous ultrasonography, CT and, more recently, MRI. However, even with modern technology, liver metastases smaller than 1 cm cannot be reliably detected by means of these techniques.[1,16] In contrast, liver metastases smaller than 0.5 cm can be seen on laparoscopy if they are located at the hepatic surface, whereas the close contact of the laparoscopic ultrasound probe with the hepatic surface allows detection of previously unknown metastases deep within the hepatic parenchyma. In the present study previously unknown liver metastases were only diagnosed by laparoscopy and confirmed by laparoscopic-guided biopsy in 12.5% of all patients. Newly detected liver metastases were particularly frequent in the subgroup of patients with advanced adenocarcinomas of the distal esophagus or cardia (22%). A similar prevalence of previously unknown hepatic metastases has also been reported in patients with advanced gastric carcinoma who are undergoing diagnostic laparoscopy.[5,7,11]

The presence of peritoneal carcinosis or malignant ascites in patients with carcinoma of the upper gastrointestinal tract is considered a contraindication for major surgical resections or neoadjuvant treatment protocols.[3,14] Peritoneal carcinosis or small amounts of malignant ascites cannot, however, be reliably diagnosed by any of the currently available imaging techniques. In the present study diagnostic laparoscopy detected previously unknown peritoneal tumor spread or free tumor cells in the abdominal cavity in 17% of patients with adenocarcinoma of the distal esophagus or gastroesophageal junction. Although the prognostic role of free tumor cells in the absence of frank peritoneal carcinosis is unclear in patients with carcinoma of the esophagus or cardia, this finding may, similar to gastric cancer, indicate early peritoneal seeding.[22]

Tumor invasion of the celiac axis lymph nodes plays a dominant prognostic role in patients with carcinoma of the esophagus or cardia.[1] Because of the inaccessibility of this area, the pretherapeutic assessment of lymph node metastases at the celiac axis has been limited and their presence or absence has so far been disregarded in therapeutic decision making. In the present study assessment of the celiac axis region with a flexible laparoscopic ultrasound probe was superior to percutaneous sonography and CT in the diagnosis of celiac axis lymph node metastasis. The accuracy of laparoscopic ultrasound in the assessment of lymph nodes may be increased even further by performing laparoscopic biopsies. Thus the status of the celiac axis lymph nodes can be included in any future treatment planning.

Although it appeared safe in the present study, diagnostic laparoscopy remains an invasive technique requiring general anesthesia and time in the operating room and, at least theoretically, carries a risk of tumor spread to the port sites and the abdominal cavity.[8,23] The clinical use of diagnostic laparoscopy should, therefore, be restricted to patients in whom the potential diagnostic gain outweighs any risk. The current study indicates that diagnostic laparoscopy provides relevant new information regarding hepatic and

peritoneal tumor spread, particularly in patients with locally advanced adenocarcinoma of the distal esophagus and cardia, whereas the diagnostic yield in patients with squamous cell esophageal cancer was low. Consequently, at our institution diagnostic laparoscopy with laparoscopic ultrasound is now mandatory in patients with locally advanced carcinoma of the distal esophagus or gastroesophageal junction who are being considered for neoadjuvant therapy or primary resection.

REFERENCES

1. Hölscher AH, Siewert JR, Fink U. Staging concepts for gastrointestinal malignancies: The importance of preoperative locoregional T- and N-staging. Gastrointest Endosc Clin North Am 1995;5:529-533.
2. DeMeester TR, Zaninotto G, Johannson KE. Selective therapeutic approach to cancer of the lower esophagus and cardia. J Thorac Cardiovasc Surg 1988;95:42-54.
3. Fink U, Stein HJ, Wilke HJ, Roder JD, Siewert JR. Multimodal treatment for squamous cell esophageal cancer. World J Surg 1995;19:198-204.
4. Stein HJ, Fink U, Siewert JR. Who benefits from combined modality treatment of esophageal carcinoma? Dis Esoph 1994;7:156-161.
5. Possik RA, Franco EL, Pires DR, Wohnrath DR, Ferreira EB. Sensitivity, specificity and predictive value of laparoscopy for the staging of gastric cancer and for the detection of liver metastases. Cancer 1986;58:1-6.
6. Warshaw AL, Gu Z-Y, Wittenberg J, Waltman AC. Preoperative staging and assessment of resectability of pancreatic cancer. Arch Surg 1990;125:230-233.
7. Kriplani AK, Kapur ML. Laparoscopy for pre-operative staging and assessment of operability in gastric carcinoma. Gastrointest Endosc 1991;37:441-443.
8. Lightdale CJ. Laparoscopy for cancer staging. Endoscopy 1992;24:682-686.
9. Bemelman WA, deWit LT, van Delden OM, Smits NJ, Obertop H, Rauws EJ, Gouma DJ. Diagnostic laparoscopy combined with laparoscopic ultrasonography in staging of cancer of the pancreatic head region. Br J Surg 1995;82:820-824.
10. Okita K, Kodama T, Takemoto O, Takeomoto T. Laparoscopic ultrasonography. Scand J Gastroenterol 1984;19:91-100.
11. Feussner H, Kraemer SJ, Siewert JR. Technik der laparoskopischen Ultraschalluntersuchung bei der diagnostischen Laparoskopie. Langenbecks Arch Chir 1994;379:248-254.
12. Goletti O, Buccianti P, Chiarugi M, Pieri L, Sbragia P, Cavina E. Laparoscopic sonography in screening metastases from gastrointestinal cancer: Comparative accuracy with traditional procedures. Surg Laparosc Endosc 1995;5:176-182.
13. Warshaw AL. Implications of peritoneal cytology for staging of early pancreatic cancer. Am J Surg 1991;161:26-29.
14. Siewert JR, Stein HJ. Adenocarcinoma of the gastroesophageal junction. Definition, classification and extent of resection. Dis Esoph 1996;9:173-182.
15. Kraemer SJM, Stein HJ, Feussner H, Siewert JR. How to do it: Extended diagnostic laparoscopy in the staging of cancer of the esophagus. Dis Esoph 1996;9:228-235.
16. Koch J, Halvorsen RA. Staging of esophageal cancer: Computed tomography, magnetic resonance imaging, and endoscopic ultrasound. Semin Roentgenol 1994;29:364-372.
17. Shandall A, Johnson C. Laparoscopy or scanning in oesophageal and gastric carcinoma? Br J Surg 1985;72:449-451.
18. Dagnini G, Caldrioni MW, Marin G, Buzzaccarini O, Tremolada C, Ruol A. Laparoscopy in abdominal staging of esophageal carcinoma: Report of 369 cases. Gastrointest Endosc 1986;32:400-402.
19. Watt I, Steward I, Anderson D, Bell G, Anderson JR. Laparoscopy, ultrasound and computerized tomography in cancer of the esophagus and gastric cardia: A prospective comparison for detecting intra-abdominal metastases. Br J Surg 1989;6:1036-1039.
20. Molloy RG, McCourtney JS, Anderson JR. Laparoscopy in the management of patients with cancer of the gastric cardia and oesophagus. Br J Surg 1995;82:352-354.
21. Siewert JR, Feussner H. Derzeitiger Stellenwert der Laparoskopie in der onkologischen Chirurgie. Dtsch Med Wochenschr 1994;119:1518-1521.
22. Bonenkamp JJ, Songun I, Hermanss J, van de Velde CJH. Prognostic value of positive cytology findings from abdominal washings in patients with gastric cancer. Br J Surg 1996; 83:672-674.
23. Childers JM, Aqua KA, Surwit EA, Hallum AV, Hatch KD. Abdominal-wall tumor implantation after laparoscopy for malignant conditions. Obstet Gynecol 1994;84:765-769.

Discussion

Dr. R. Hinder (Omaha, Neb.). I would like more information on the true positive and true negative findings with respect to the liver. How often did you suspect there was liver metastasis, and you never detected it on biopsy, or vice versa? How often did you not see it and later, in the course of the patient's development of the disease, did you find that you had missed a liver metastasis? Furthermore, you never explained how you identified the true and false positives for the lymph nodes. Did you finally biopsy these lymph nodes so you could examine them microscopically?

Dr. H.J. Stein. The study was designed so that none of the liver metastases that I have presented had been suspected on our standard staging modalities. If we had a patient who, on CT or percutaneous ultrasound, had a suspected liver metastasis, that patient would have undergone percutaneous biopsy, CT, or ultrasound-guided imaging and would not have been included in this study. None of the cases of liver metastases presented here, a total of 16 in the 127 patients, were suspected on CT scan or percutaneous ultrasound.

All of our newly detected metastases were confirmed by means of laparoscopically controlled biopsy. All of the data presented are histologically documented metastases. Thus all of our positive findings are true positives as far as liver mestastases are concerned.

The analysis of lymph nodes was based on patients who subsequently had surgery and lymphadenectomy in this area. Surgical removal of the lymph nodes and histopatho-

logic assessment of these lymph nodes served as the "gold" standard. The sensitivity and specificity were calculated based on surgical and histopathologic standards. We did not perform laparoscopic biopsy of lymph nodes at the celiac access site for fear of tumor spread.

Dr. J. Peters (Los Angeles, Calif.). The key question limiting the use of diagnostic laparoscopy is, what are you going to do with the information that you gain and how will it alter your treatment plan? You have chosen to pursue "palliative therapy" in patients who have positive findings. Does that include transhiatal esophagectomy in your hands? I think most of us believe that esophagectomy is still the best form of palliation.

Also, what is the "clinical value," as your title would suggest, of positive peritoneal washings or perhaps even celiac nodes, or a 5 mm hepatic metastasis? If you endorse transhiatal esophagectomy for palliation, then why not stage patients at the time of laparotomy, pursuing a curative resection in those that have negative operative findings and a palliative resection in the remainder?

Finally, what is gained by the use of laparoscopic ultrasound? As you may know, data from our institution suggest that celiac metastases do not preclude long-term survival, which makes the detection of small liver metastases really its only use. Would not the use of better imaging techniques such as CT portography be a less invasive means?

Dr. Stein. Laparoscopy does alter our treatment. This is important to emphasize because this invasive staging modality does only make sense if it has an influence on decisions regarding therapy.

In our hands the documentation of liver metastases, as small as you want, 0.5 mm or smaller, or the documentation of peritoneal tumor spread would exclude the patient from a curative approach. We do not treat patients with systemic disease such as liver metastasis or peritoneal tumor spread by means of transhiatal resection. Our colleagues in internal medicine and gastroenterology have several noninvasive therapeutic options available for these patients. We tend to treat these patients palliatively using laser, stents, or percutaneous radiation. We do not perform palliative resections because of the associated high morbidity and mortality and the availability of less invasive and equally effective palliative options.

What is the clinical importance of free tumor cells? We have previously shown that free tumor cells in the abdominal cavity, shown by abdominal lavage, are equivalent to peritoneal tumor spread. The prognosis for these patients is poor and cannot be improved by either chemotherapy or surgical intervention. We offer palliation if they have dysphagia, by means of laser therapy or stents, but we do not consider any type of aggressive surgical treatment if we find free tumor cells in the abdominal cavity.

Why do we not perform staging at the time of laparotomy? We would like to offer patients the potential benefits of neoadjuvant modalities. Diagnostic laparoscopy is invasive, but it is less invasive than a diagnostic laparotomy.

What is the prospect of other staging modalities? Of course, with high-resolution positron emission tomography, MRI, and CT portography, this study probably will have to be redone. We are currently using the standard staging techniques, that is, CT with intravenous and oral contrast and percutaneous ultrasound, which today represent the available staging modalities at most community hospitals.

Dr. J. Hunter (Atlanta, Ga.). In your Methods section, you suggested that there would be some benefit to opening and exploring the lesser sac, and yet you told us very little about that. Were there any benefits to performing that exploration, and what are your feelings now about the utility of lesser sac exploration in laparoscopic staging of esophageal cancer?

Dr. Stein. We found peritoneal tumors only in the lesser sac in three of our patients. These would have been missed if we had not opened the sac.

Dr. E. van Dykum (Amsterdam, The Netherlands). What was the additional value of laparoscopic ultrasonography in these patients?

Dr. Stein. Most of the liver metastases were detected by laparoscopic ultrasonography alone. Only 2 of the 16 cases of newly detected liver metastases were found on direct inspection; thus 14 of the 16 cases of newly detected liver metastases were noted only on laparoscopic ultrasonography.

Receptor-Dependent Growth Inhibition of Human Pancreatic Cancer by 9-*cis* Retinoic Acid

S.M. Vickers, L.K. Sampson, W. Ying, J.O. Phillips

Pancreatic cancer continues to be a lethal disease and ranks as the fifth major cause of cancer death with a 2-year survival rate of only 8%. Although significant progress has been made in the surgical management of this malignancy, there have been only minimal advances in adjuvant therapy. Based on the lack of effective adjuvant or primary therapy for these patients, we tested the effects of various retinoids (all-*trans*, 9-*cis*, and 13-*cis* retinoic acids) on the growth of several human pancreatic cancer cell lines. Four human pancreatic cancer cell lines, designated PANC-1, ASPC, BxPc, and HPAF, were studied. Three types of retinoic acid were added to subconfluent monolayers of the different cancer cell lines over a range of concentrations (1 to 20 μmol/L). Effects on cell growth were determined daily over 96 hours by a cell proliferation assay (MTT). Nuclear receptor (RAR/RXR) transcript and protein were determined by reverse transcription polymerase chain reaction and Western blot analyses. Three (PANC-1, ASPC, and BxPc) pancreatic cancer cell lines responded in a dose-dependent fashion with a significant decrease in cell growth at clinically relevant concentrations of 9-*cis* retinoic acid (7.5 to 10 μmol/L). All-*trans* and 13-*cis* retinoic acid did not affect cell growth in the four pancreatic tumors. (J GASTROINTEST SURG 1997; 1:174-181.)

Adenocarcinoma of the pancreas is currently the fourth or fifth most common cause of cancer death in the United States. The incidence has continued to increase with some 28,000 new cases per year; only 8% of these patients are still alive at 2 years.[1] Yet a great deal of progress has been made in the surgical treatment of this disease, with most academic centers reporting a perioperative mortality rate of 5% or less.[2,3] However, despite the significant improvement in surgical treatment, long-term survival has not improved.[4] Given that the majority of patients are not treatable by operative resection and chemotherapy alone is relatively ineffective in treating advanced pancreatic cancer, new therapeutic strategies are needed if we are to attain significant improvement in patient survival.

Retinoids offer strategies that focus on a parallel induction of tumor differentiation and inhibition of tumor cell proliferation.[5,6] Vitamin A (retinol) and its naturally occurring derivatives, all-*trans* retinoic acid (ATRA), 3,4-didehydroretinoic acid, and 9-*cis* retinoic acid (9-*cis* RA) have demonstrated their effectiveness in numerous studies by inhibiting a variety of malignancies in experimental tumor models.[7-10] Over the past several years much has been learned concerning the mechanisms by which retinoids exert their growth-inhibiting effect and induce tumor cell differentiation.[11] Currently we know of two families of nuclear retinoic acid (RA) receptors, each consisting of three isoforms: α, β, and γ. Both retinoic acid receptors (RARs) and retinoic X receptors (RXRs) are ligand-inducible transregulators of transcription activity initiated from RA promoter targeted DNA.[5,12-14] These two classes of receptors (which belong to a larger superfamily of steroid receptors) mediate the actions of endogenous retinoic acids. The characteristic feature of these receptors is that they bind to DNA regulatory regions called retinoic acid response elements and activate transcription in a ligand-dependent manner.[15,16] Within the nuclear receptors there are several structural domains. In particular there is a ligand-binding domain (region E) at the carboxyl terminus, which controls not only ligand binding but nuclear translocation, dimerization, transactivation, and interaction with heat shock proteins. The conserved DNA binding domain (region C) has several cysteine residues coordinated by zinc "fingers"

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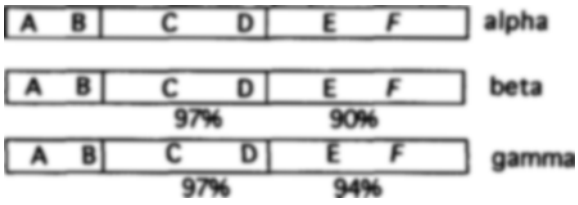

Fig. 1. Schematic of RA receptor structural genetic domain. Retinoid action is mediated by two families of receptors—retinoic acid receptors (RARs) and retinoic X receptors (RXRs)—each with three subtypes: alpha, beta, and gamma. Each receptor with its domain A-F for RARs and A-E for RXRs with the number of amino acids ranging from 410 to 467.

that tightly bind RAR and RXR response elements. The function of regions D and F is unknown (Fig. 1). These nuclear receptors bind as dimers with the favored dimeric species, an RXR/RAR heterodimer.[5] The heterodimer, through an intrinsic activation region referred to as AF-2 (region E) (located in the ligand-binding domain), translates the ligand-binding signal to transcriptional activity.

The naturally occurring ligand for RARs in vivo is ATRA, whereas the RXRs preferentially bind to 9-*cis* RA.[15-17] The diversity of the biologic processes affected by RA as well as the tissue-specific expression of RAR/RXR subtypes suggest that each subtype has a specific function, thereby determining whether any given cell is retinoid sensitive or resistant.[16] However, it is now known that RAR β acts as a tumor suppressor gene in human epidermoid small cell lung cancer and that the t(15:17) translocation breakpoint of acute promyelocytic leukemia lies within the RAR-χ locus.[10,18] This latter finding has allowed clinical treatment with ATRA in patients with acute promyelocytic leukemia, thereby inducing leukemia cell differentiation and achieving remission in a large proportion of patients.

At the present time little is known about the effects of retinoids on pancreatic cancer. In this study we have evaluated the potential therapeutic role of retinoids in ductal pancreatic cancer and characterized the elements responsible for this response.

MATERIAL AND METHODS

Cell Culture

All pancreatic cancer cell lines (PANC-1, ASPC, BxPc, and HPAF) were obtained from American Type Culture Collection (Rockville, Md.). These cell lines were maintained in RPMI-1640 medium supplemented with L-glutamine (200 mg/ml), penicillin (100 mg/ml), streptomycin (25 mg/ml), and 10% heat-inactivated fetal bovine serum (FBS) at 37° C in a humidified 5% carbon dioxide atmosphere.

Growth Inhibition Assays

Cell lines were grown to confluence in 100 mm flasks, and 0.25% trypsin (Gibco, Grand Island, N.Y.) was subsequently added. Cells (5000) were plated in 96 well plates in RPMI-1640 medium supplemented with 10% FBS. These cells were allowed to adhere for 24 hours, after which the medium was removed and complete media (RPMI-1640 with 10% FBS), with graduated concentrations (2.5, 5, 7.5, and 10 mmol/L) of 9-*cis* RA, ATRA, and 13-*cis* RA (the stereoisomer of ATRA) (Sigma Chemical Co., St. Louis, Mo.) were dissolved in 0.1% dimethyl sulfoxide (DMSO). Cells were also grown in RPMI-1640 with 10% FBS in 0.1% DMSO as a control. These cells were counted using the MTT assay (Promega Corp., Madison, Wis.) (which measures intracellular dehydrogenases) at 96 hours. Viability was determined by trypan blue exclusion.

Reverse Transcriptase–Polymerase Chain Reaction

Reverse transcription of RNA from cell lines was performed using 1 μg total RNA, 100 pmol/L random hexamer primer, 1 mmol/L, dithiothreitol 6 mmol/L Mg^{2+}, 500 μmol/L of each deoxynucleoside triphosphate, and 20 U RNAsin (Promega Corp.) and Moloney murine leukemia virus reverse transcriptase. The reverse transcriptase mixture was used directly as a template for the polymerase chain reaction (PCR) in a dilution of 1:20. For amplification the following receptor subtype–specific 5′- and 3′-primers were designed complementary to the human or mouse nucleotide sequences:

Human RAR α,
5′-TGGGT,GGACT,CTCCC,CGCCA
(5′-primer) and
5′-CAC-GC,TGACG,CCGGA,GGTGG,G
(3′-primer)
Human RAR β
5′-CACTG,GCTTG,ACCAT,CGCAG,ACC
(5′-primer) and
5′-CGTGC,AGCTG,GATCT,GGGGC,TG
(3′-primer)
Human RXR α,
5′-ATGGC,TGCCC,CCTCG,CTGCA,C
(5′-primer) and
5′-GGCGC,AGATG,TGCTT,GGTG
(3′-primer)

Human RXR β,
5′-ATGCC,ACCCC,CGCCA,CTGGG,C
(5′-primer) and
5′-GCCTC,CAGGA,TCCTG,TCCAC,AGGC
(3′primer) and
Mouse RXR γ,
5′-CCCCT,GGTCA,CACTG,GCTCG,ACG
(5′-primer) and
5′-CACCA,GAGAC,CCAGG,GCTGG,TGG
(3′-primer).

The expected molecular size of the PCR amplification products was as follows (in base pairs): RAR α, 438; RAR β, 435; RAR γ, 515; RXR α, 327; RXR β, 552; and RXR γ, 351. The reaction was performed in 10 mmol/L Tris-HC1 buffer (pH 9.0) containing 50 mmol/L KC1, 0.01% Triton X-100, 1 mmol/L $MgCl_2$, 200 μmol/L of each deoxynucleoside triphosphate, 50 pmol/L of each primer, and 2.5 U *Thermus aquaticus* DNA polymerase in a final volume of 50 μl. Polymerase chain reaction for the human RAR a was performed in the same buffer except for an $MgCl_2$ concentration of 1.5 mmol/L. Amplification conditions for 35 cycles were performed as follows: denaturation for 30 seconds at 92° C, annealing at 60° C for 90 seconds, and extension for 90 seconds at 72° C with an increase of 5 seconds each cycle. An extension was performed for an additional 10 minutes after completion of all 35 cycles. For each experimental condition, one RNA aliquot was amplified without having been subjected to the reverse transcription PCR. This internal control was required to ensure that the observed PCR product was not due to amplification of contaminating genomic DNA.

Western Blot Analysis

Pancreatic cancer cell lines in 100 mm flasks were grown to confluence, trypsinized (0.25%), lysed in 1 × RIPA buffer (1% NP-40, 0.5, sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mmol/L Tris-HCE, 150 mmol/L NACE, 1 mmol/L Na^2-EDTA, and 0.1 mmol/L EGTA), and spun 10,000 × *g* for 20 minutes. The supernate was removed and assayed for protein concentration (Bio-Rad Laboratories, Life Science Group, Hercules, Calif.), and 30 μg of protein was combined with 5 × SDS - page sample buffer - and run on 8% SDS - polyacrylamide gel at 100 volts for 2½ hours. Proteins were then transferred to a nitrocellulose membrane (0.45 mm) overnight at 40 volts. Nonspecific binding sites were blocked by incubating them with 5% nonfat dried milk in phosphate-buffered saline (PBS) solution for 3 hours.

Blots were washed three times in PBS and incubated with primary antibody RAR β (polyclonal antibody) or RXR β (monoclonal antibody) from Affinity Bioreagents (Golden, Colo.) in PBS plus 0.05% Tween 20 solution at 1:2000 dilution overnight (18 hours). The blots were washed three times with PBS plus 0.05% Tween 20 and two antibodies—a biotinylated goat antimouse or goat antirabbit IgG antibody—was applied at 1:30,000 dilution for 3 hours. The blots were washed three times with PBS or Tris plus 0.05% Tween 20 using six changes over 1 hour. The blots were developed with the Vectastain ABC development kit (Vector Laboratories, Inc., Burlingame, Calif.) and with the enhanced chemiluminescence kit (Amersham Corp., Arlington Heights, Ill.).

Statistical Analysis

Statistical analysis of the growth inhibition curves were performed by means of the Wilcoxon rank-sum test.

RESULTS

Growth Inhibition Studies

To initially determine the antiproliferative effect of retinoids in vitro, we examined the following four human pancreatic ductal carcinoma cell lines: PANC-1, ASPC, BxPc, and HPAF. Our initial studies involved both ATRA and 13-*cis* RA, with neither demonstrating growth inhibition in any of the four cell lines in the physiologic dosage range (5 to 10 mmol/L) (only the BxPc cell line shown) (Fig. 2)[19] However, our application of 9-*cis* RA consistently demonstrated significant growth inhibition in all of the cell lines except HPAF at 7.5 and 10 mmol/L, when assayed at 72 and 96 hours $P < 0.01$ (see Fig. 2).

Expression of Nuclear RARs and RXRs in Pancreatic Carcinoma Cell Lines

Reverse transcription of the retinoic receptors, using oligonucleotide primer (isoform specific) complementary to the human nucleotides, detected expression of the mRNA message for the γ isoform of the RAR but not the α and β isoforms, which were not detected at 28 or 35 cycles in any of the four human lines. This RAR γ isoform has been identified and previously described as a 515 base pair (bp) band, identical to the band seen in this study. Next, expression of the RXR subtypes was identified in two of the three isoforms (α and β but not γ) in each of the four lines (Fig. 3). These isoforms were identified at their characteristic bands, α isoform at 327 bp and the RXR β isoform at 552 bp.[19]

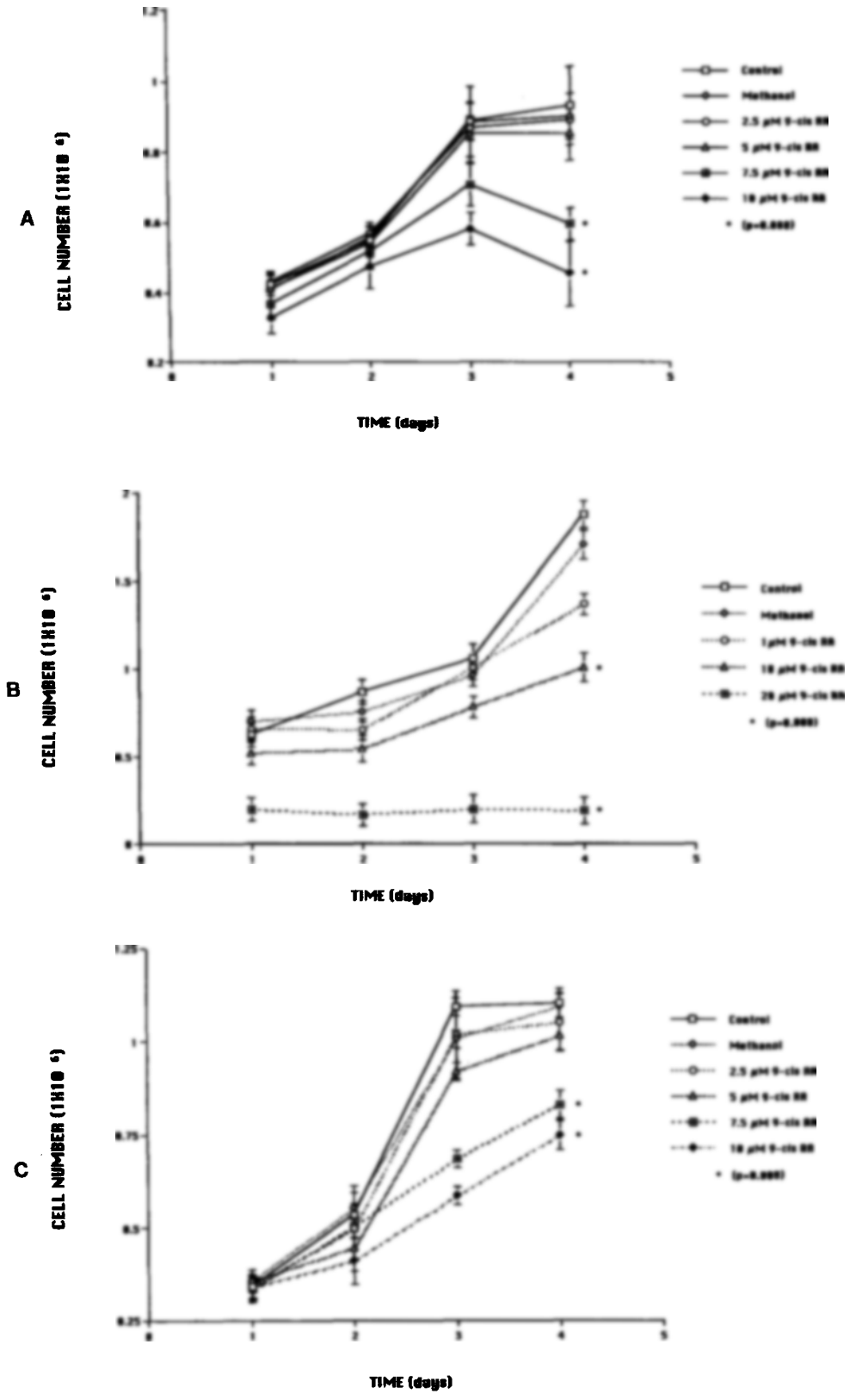

Fig. 2. Growth inhibition curves for 9-*cis* RA in four pancreatic cancer cell lines (**A,** ASPC; **B,** BxPc; and **C,** PANC-1.

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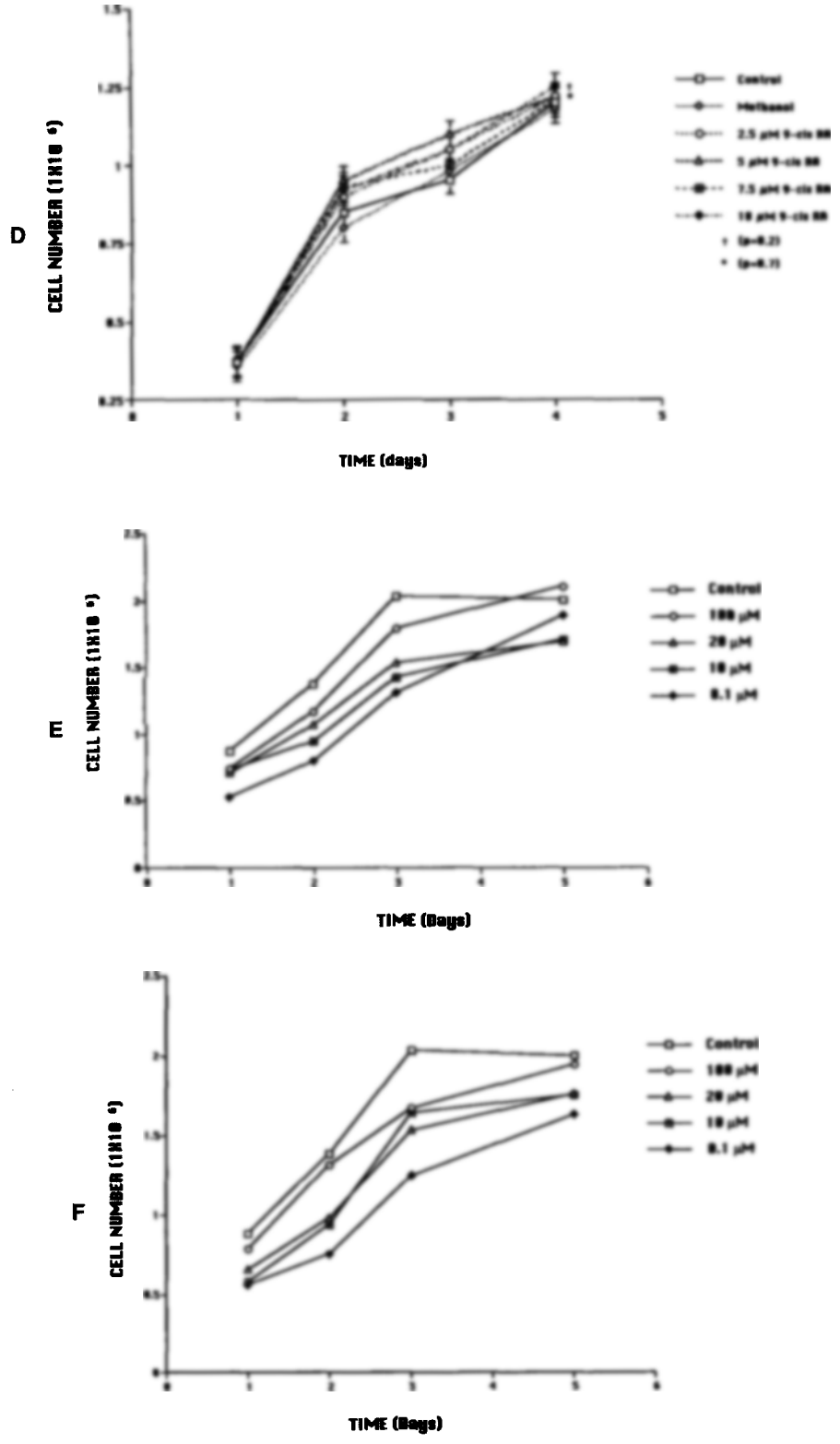

Fig. 2, cont'd. D, HPAF). Each line was tested in triplicate; 5000 cells were seeded in 96 well plates, incubated with varying concentrations of 9-*cis* RA (no response was seen, even at the highest dose, in 13-*cis* RA or ATRA), and assayed at 24-hour intervals for 4 days. Each cell line demonstrated a significant inhibitory response to 7.5 and 10 mmol/L of 9-*cis* RA by day 3 or 4 in all cell lines except HPAF; 20 mmol/L of 9-*cis* RA was toxic to all cell lines. Inhibition of the BxPc cell line with ATRA **(E)** and 13-*cis* RA **(F)** is also shown. Each of the cell lines failed to respond at similar (physiologic) concentrations of ATRA. BxPc cell line is shown as a representative example.

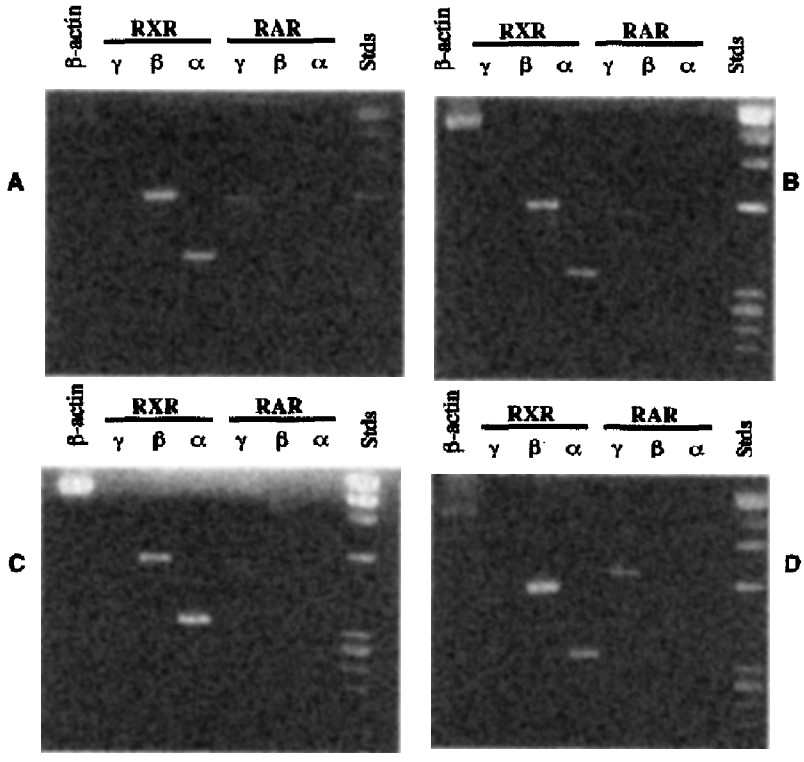

Fig. 3. Reverse transcription polymerase chain reaction (PCR) for RXR and RAR isoforms. Total RNA (1.0 mg) isolated from several confluent monolayers of each cell type (**A**, PANC-1; **B**, ASPC; **C**, BxPc; and **D**, HPAF) was used in reverse transcription PCR analysis (35 cycles) with isoform specific sense and antisense primer. Amplification products were separated on 2% weight/volume agarose gel and stained with ethidium bromide. Each gel contained a 1.0 kilobase pair DNA ladder, which served as a molecular mass marker (*Stds*). **A**, Reverse transcriptase analysis of the PANC-1 cell line labeled from left to right demonstrating characteristic products for RXR β552 bp α327 bp and for RAR γ515 bp. This same configuration of products is seen for each of the other cell lines (**B**, **C**, and **D**).

Western Blot Analysis

Western blot analysis with commercially available antibodies, RAR γ (polyclonal antibody), 1:2000 dilution, and RXR β (monoclonal antibody), confirmed the presence of RAR γ receptor protein as evidenced by a 52 kd protein in all four cell lines (Fig. 4). However, the presence of the RXR β receptor was found to be expressed in only three (ASPC, PANC-1, and BxPc) of the four lines, which responded to 9-*cis* RA growth inhibition (see Fig. 4). This protein RXR β (which binds only 9-*cis* RA) was not found in the HPAF cell line, which failed to respond to physiologic doses of 9-*cis* RA.

DISCUSSION

The majority of patients diagnosed as having pancreatic adenocarcinoma present with locally advanced disease. To date, highly aggressive chemotherapy approaches have had only a minimal impact on survival. Recently retinoids have been used to treat a variety of malignancies with the presumed mechanism of action being induction of cellular differentiation and inhibition of tumor cell growth. This occurs through ligand binding to specific nuclear retinoic acid receptor (RARs and RXRs) interactions, which in turn control the transcription of genes via interaction with specific DNA sequences, RA response elements.[20] These *cis*-acting DNA[5] response elements have previously been shown to activate and modulate a number of transcription target genes and resulting gene products for basement membrane proteins such as laminin B_1 and collagen type IV.[5] These products can certainly play a vital role in cell infrastructure and elements critical to cellular and organ cytoskeleton structure. Our data support the notion that the critical point of cellular sensitivity is the presence of nuclear receptors. All four of the cell lines appear to express the message of one or more isoforms from each of the receptor groups. The significance of this may be reflected in the lack of

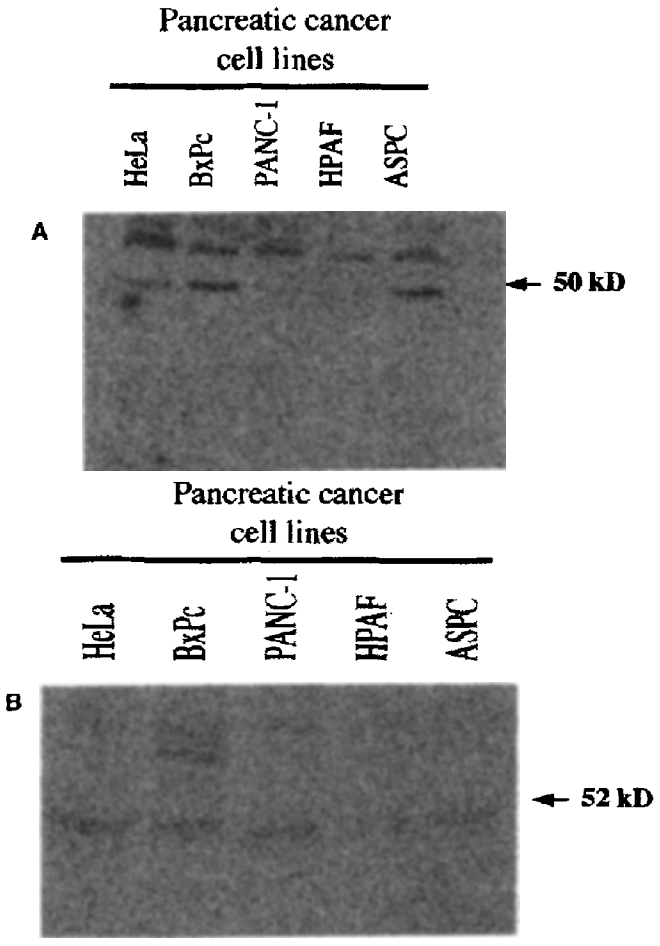

Fig. 4. Western blot analysis of RXR and RAR receptor proteins. Extracted proteins were harvested from total cellular extract from all four cell lines (1×10^6 cells per lane). **A,** Samples were analyzed (run on 10% weight/volume reducing SDS-page polyacrylamide gel) by affinity purified monoclonal antibody against RXR β, demonstrating the characteristic 50 to 55 KD band for the RXR β complex, the presence of which is seen in all cell lines except HPAF (a faint band is seen in the PANC-1 cell line). **B,** Anti-RAR γ polyclonal antibody demonstrates a 52 KD band in all four of the pancreatic cell lines consistent with the RAR γ receptor.

growth inhibition in these tumors to physiologic concentrations of ATRA and 13-*cis* RA; this contradicts previously reported data[19] on the response of pancreatic cancer to RA. This study by Rosewicz et al.[19] found a broad uniform response to ATRA in all of the human cancer cell lines tested but not in the rat acinar cell line AR 42J. However, the cellular components of this response were primarily evaluated at the mRNA (message) level, which does not guarantee protein expression. Our studies show that only the message for the RAR γ isoform was found with no evidence of the α and β isoforms. Western blot analysis for RAR γ demonstrated the presence of this receptor in all of the cell lines. Messenger RNA for the RXR nuclear receptors identified both β and α isoforms. Western blot analysis with an anti-RXR β monoclonal antibody confirmed the presence of the receptor in all of the 9-*cis* RA responsive cell line but not HPAF. This is significant in that the preferred in vivo ligand for RXRs is 9-*cis* RA, the only RA derivative that produced a statistically significant growth inhibition in three of the four cell lines, which also expressed the RXR β receptor protein. The lack of a response in the HPAF cells, which failed to respond to either ATRA or 13-*cis* RA (the stereoisomer of ATRA), was consistent with observations in other cell lines. However, in contrast to the other cell lines that exhibited a significant response at 7.5 and 10 mmol/L of 9-*cis* RA, this cell line demonstrated no growth inhibition at either dose. This biologic response, or lack of growth inhibition, may be explained by the absence of the RXR β receptor protein, which may be essential for 9-*cis* RA to achieve its antiproliferative effect in pancreatic cancer. Clearly, further characterization of each of the nuclear receptor proteins is needed in addition to evaluation of the presence and level of cellular RA binding proteins, which are thought to control the availability of free RA within the cell.[20,21] In vivo studies must also be addressed in order to evaluate the efficacy of these preparations. This remains somewhat of a challenge since the normal route for RA is oral and bioavailability is largely dependent on oral food intake, but an intraperitoneal model is currently being evaluated for in vivo efficacy. The fact that 9-*cis* RA demonstrated growth inhibition in three of the four pancreatic tumor cell lines is encouraging since this inhibition appears to be related to expression of the 9-*cis* RA ligand-specific RXR β–receptor protein. Finally, this may lead to opportunities for clinical therapeutic intervention for pancreatic cancer as well as possibly allowing gene therapy intervention for manipulation of specific nuclear receptors to further potentiate the therapeutic effects of RA in these types of tumors.[22]

REFERENCES

1. Lillimoe KD. Current management of pancreatic carcinoma. Ann Surg 1995;221:133-148.
2. McGuire GE, Pitt HA, Lillimoe KD, et al. Reoperative surgery for periampullary adenocarcinoma. Arch Surg 1991; 126:1205-1212.
3. Crist DW, Sitzmann JV, Cameron JL. Improved hospital morbidity, mortality and survival after the Whipple procedure. Ann Surg 1987;3:358-365.
4. Gudjonsson B. Carcinoma of the pancreas: Critical analysis of cost, results of resection and the need for standardized reporting. J Am Coll Surg 1995;181:483-503.
5. Pemrick SM, Lucas DA, Grippo JF. The retinoid receptors. Leukemia 1994;8 (Suppl 3):S1-S10.

6. Connie M, Agadir A, Degos L, Chomienne C. Retinoids and differentiation treatment: A strategy for treatment in cancer. Anticancer Res 1994;14:2339-2346.
7. Butler WB, Fontant JA. Responses to retinoic acid of tamoxifen-sensitive and resistant sublines of human breast cancer cell line MCF-7. Cancer Res 1992;52:6164-6167.
8. Eisenhauer EA, Lippman SM, Kavanagh JJ, et al. Combination 13-*cis* retinoic acid and interferon α 2a in the therapy of solid tumors. Leukemia 1994;8:1622-1625.
9. Adamson PC. Clinical and pharmacokinetic studies of all-trans retinoic acid in pediatric patients with cancer. Leukemia 1994;8:1813-1816.
10. Houle B, Rochette-Egly C, Bradley WEC. Tumor-suppressive effect of the retinoic acid receptor β in human epidermoid lung cancer cells. Proc Natl Acad Sci USA 1993;90:985-989.
11. Chambon P. The molecular and genetic dissection of the retinoid signaling pathway. Recent Prog Horm Res 1995; 50:317-332.
12. Berroden TJ, Marks MS, Ozato EL, et al. Heterodemerization among thyroid hormone receptor, retinoic acid receptor, retinoic X receptor, chicken ovalbumin upstream promoter transcription factor, and endogenous liver protein. Mol Endocrinol 1992;6:1468-1478.
13. Leid M, Kastner P, Lyons R, et al. Purification, cloning, and RXR identity of the *Hela* cell factor with which *RAR* or *TR* heterodimerizer to bind target sequence efficiently. Cell 1992;68:377-395.
14. Brand N, Petrovich M, Krust A, et al. Identification of a second human retinoic acid receptor. Nature 1988;332:850-853.
15. MacDonald PN, Dowd DR, Nakajima S, et al. Retinoid X receptors stimulate and 9-*cis* retinoic acid inhibits 1, 25-dehydroxy vitamin D_3-activated expression of the rat osteocalan gene. Mol Cell Biol 1993;13:5907-5917.
16. Leid M, Kastner P, Chambon P. Multiplicity generates diversity in the retinoic acid signaling pathways. Trends Biol Sci 1992;17:427-433.
17. Heyman RA, Mangelsdorf DJ, Dyck JA, et al. 9-*cis* Retinoic acid is a high affinity ligand for the retinoic X receptor. Cell 1992;68:397-406.
18. Mundi JRF, Frankel SR, Huselton C, et al. Clinical pharmacology of oral all-*trans* retinoic acid in patients with acute promyelocytic leukemia. Cancer Res 1992;52:2138-2142.
19. Rosewicz S, Stier U, Brembeck F, et al. Retinoids: Effects of growth differentiation and nuclear receptor expression in human pancreatic carcinoma cell lines. Gastroenterology 1995;110:1646-1660.
20. Gigurre V. Retinoic acid receptors and cellular retinoic binding proteins: Complex interplay in retinoic signaling. Endocr Rev 1994;94:61-79.
21. Wei LN, Mertz JR, Goodman DS, et al. Cellular retinoic acid and cellular retinal-binding proteins: Complementary deoxyribonucleic acid cloning, chromasomal assignment and tissue specific expression. Mol Endocrinol 1987;1:526-534.
22. Libby PR, Bertram JS. Lack of intracellular binding proteins in a retinol-sensitive cell line. Carcinogenesis 1982;3:481-484.

Discussion

Dr. R.H. Bell, Jr. (Seattle, Wash.). I wonder whether there are examples in other better studied types of tumors, whether the absence of these nuclear receptors for RA is associated with a change in the biology of the tumor. Have you had the opportunity to perform PCR analysis on any fresh pancreatic cancer specimens and determine whether you can detect the absence of receptor expression in operative specimens and correlate that with the extent of differentiation or survival?

Dr. S.M. Vickers. It is believed that in small cell epidermoid cancer of the lung the lack of RAR β is a tumor suppressor gene, so the lack of that receptor, in fact, leads to overgrowth of that tumor. We have some primary cells that we are going to examine for expression, or lack of expression, of those receptors to determine whether they in fact function as a tumor suppressor gene.

Dr. Bell. You stated that RA has an effect on the synthesis of basement membrane proteins. Does it have any effect on matrix and the development of metastases?

Dr. Vickers. I do not know. I would like to think it does because it may play some role in metastasis. It clearly has some effect on structural proteins.

Dr. L. Boros (Columbus, Ohio). What was the method you used to count the cells?

Dr. Vickers. The MTT assay (a cellular dehydrogenase based assay) is used like an enzyme-linked immunosorbent assay; it is a colorimetric assay so one is measuring against a standard curve of viable cells with a known quantity. A specific color is obtained for a count for a known number of cells.

Dr. Boros. Is the retinoic acid already in the system when you add the formazan dye? We had a problem with that assay. The formazan accumulated and it was turned into a dye related to mitochondrial function. Any agent that affects the metabolism of the cell will significantly change the outcome of the assay. I would suggest you consider this in future experiments.

Dr. D. McFadden (Los Angeles, Calif.). In the breast cancer literature it appears that these agents are more effective in chemoprevention than therapy. Have you considered using them in the tumor genesis model in pancreatic cancer.

Dr. Vickers. That certainly would be an area in which RA may be seen to play a more significant role. We have not attempted to use it in a model yet, and I think probably its ultimate role may be that as opposed to treatment.

Long-Term Outcome of Completion Gastrectomy for Nonmalignant Disease

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Between 1989 and 1995 we performed completion gastrectomy for non-malignant disease in 21 patients (11 men and 10 women, mean age 48.4 years). These patients had undergone a total of 48 prior gastric operations. Indications for completion gastrectomy in this group were anastomotic ulceration with stricture in eight patients, alkaline reflux gastritis and/or esophagitis in eight, postsurgical gastroparesis in two, gastroesophageal necrosis in two, and gastrocutaneous fistula in one. Major preoperative symptoms included nausea and vomiting in 16 cases, abdominal pain in 15, dysphagia in 14, heartburn in seven, and weight loss in five. Following completion gastrectomy, five patients (24%) had serious complications and there was one postoperative death (5%). Five patients were lost to follow-up. For the remaining 15 patients, mean follow-up has been 30 months with a range of 1 to 70 months. These patients were all interviewed and eight (53%) report significant improvement, two (13%) report moderate improvement, and four (27%) report no improvement; one patient (7%) has had worsening of symptoms since undergoing completion gastrectomy. The average body weight index was essentially unchanged after completion gastrectomy. We conclude that completion gastrectomy with Roux-en-Y esophagojejunostomy results in a favorable outcome in the majority of selected patients with diseases of the foregut who are unresponsive to less radical treatment. (J GASTROINTEST SURG 1997;1:182-187.)

Total gastrectomy has traditionally been considered a radical procedure because it was initially associated with a relatively high operative mortality and morbidity. Although postoperative sequelae such as dumping syndrome and chronic diarrhea occur with similar frequency after subtotal gastrectomy, patients undergoing gastrectomy have been particularly prone to malnutrition and weight loss, and total gastrectomy has been utilized only in patients with gastric carcinoma. Initially, total gastrectomy was used primarily for "curative resections" in patients with gastric carcinoma because many believed that the resultant metabolic abnormalities precluded its use as a palliative procedure.[1] Because many of these patients had a recurrence of the carcinoma and died within a relatively short period of time after surgery, it was difficult to evaluate the effect of total gastrectomy on the quality of life.

In the 1960s and 1970s total gastrectomy was frequently used as an effective method of obliterating hyperacidity in patients with Zollinger-Ellison syndrome.[1-5] Because these patients had either benign tumors or low-grade malignancies, their nutritional status could be assessed independently from their tumors. Patients who underwent total gastrectomy for Zollinger-Ellison syndrome did well in terms of nutrition or weight maintenance provided that bile was diverted from the esophagus. A Roux-en-Y esophagojejunostomy with diversion of the bile 40 cm distally has, therefore, become the standard method of bile diversion following total gastrectomy.[1-6] Some surgeons prefer to fashion a jejunal pouch at the esophageal anastomosis, whereas others perform standard end-to-side esophagojejunostomy. The critical factor in reconstruction, however, is diversion of bile from the esophagus rather than construction of a particular pouch.

The lack of serious nutritional sequelae in patients with Zollinger-Ellison syndrome treated with total gastrectomy has prompted surgeons to use total gas-

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trectomy to treat such benign diseases as recurrent peptic ulcer following several ulcer operations, postsurgical gastroparesis, chronic gastric atony, and multiple failures of antireflux operations. Although total gastrectomy is currently being used to treat several nonmalignant conditions, there is a relative paucity of data on the long-term morbidity of this procedure in these patients. We evaluated nutritional outcomes and long-term patient satisfaction in a series of patients who underwent total gastrectomy for nonmalignant disease.

MATERIAL AND METHODS

Between 1989 and 1995, a total of 21 patients underwent completion gastrectomy for benign disease at Oregon Health Sciences University Hospital and at Portland Veterans Affairs Medical Center. This included 11 men and 10 women whose average age was 48.4 ± 13 standard deviation years (range 26 to 76 years).

Four patients were lost to follow-up and two have since died. The remaining 15 patients were contacted by phone or seen and evaluated in person. They were questioned regarding their general health and any specific gastrointestinal complaints. They were asked about their eating habits and any dietary restrictions. In addition, blood samples were obtained from most of them for determination of albumin, hematocrit, and total protein levels.

Patients were also weighed and this weight was compared with the preoperative weight. In addition to absolute weight, we calculated the body weight index (BWI), which is a better indicator of nutritional status. BWI was calculated by dividing the body weight in kilograms by the square of height in meters: BWI (kg/m^2) = weight (kg)/height^2 (m). BWI of 20 kg/m^2 is considered a level above which patients are considered to be receiving adequate nourishment and below which they are considered to be malnourished.

RESULTS

Twenty-one patients underwent completion gastrectomy. Two required esophagectomy with right colon interposition. This group of 21 patients had 48 operations before undergoing completion gastrectomy. The operations are listed in Table I. The indications for completion gastrectomy are presented in Table II. There were five major complications (24%) including two cases of intra-abdominal abscesses. One patient was returned to the operating room for drainage of the abscess and one was treated with percutaneous drainage. In a third patient, multiple abdominal abscesses were due to anastomotic leakage and resulted in peritonitis, sepsis, and eventually death. This was the only operative death (5%) and it occurred 40 days after completion gastrectomy. Other complications included postoperative pneumonia, which resolved with antibiotic treatment, and one case of respiratory distress, which required a short period of intubation and mechanical ventilation. The last complication was pseudomembranous colitis, which was initially misdiagnosed and attributed to dumping syndrome. The correct diagnosis was eventually made and the patient was treated with oral vancomycin, after which her symptoms rapidly resolved.

All patients underwent routine postoperative gastrografin swallow examination approximately 1 week postoperatively to evaluate the integrity of the anastomosis. Only one patient showed radiographic evidence of a leak (one of the two patients with colon interposition), and this was treated by withholding oral intake for one additional week, at which time results of repeat Gastrografin study were normal. There were two other documented cases of anastomotic leakage. One occurred in a patient who presented 20 days after surgery complaining of fever, chills, and abdominal pain. Repeat Gastrografin swallow test revealed a small leak, which was successfully treated nonsurgically with total parenteral nutrition, intravenous antibiotics, and restriction of oral intake for 1 week. The other leak occurred in the one patient who died postoperatively. This patient was seen at another

Table I. Surgical procedures prior to completion gastrectomy

Vagotomy and antrectomy with Billroth I	6
Vagotomy and antrectomy with Billroth II	10
Roux-en-Y gastrojejunostomy	5
Partial gastrectomy	2
Vagotomy and pyloroplasty	3
Nissen fundoplication	12
Gastric banding or stapling for weight loss	5
Other	5
TOTAL	48

Table II. Preoperative diagnosis and indications for completion gastrectomy

Anastomotic ulcer with stricture	8
Recurrent reflux gastritis/esophagitis	8
Postsurgical gastroparesis	2
Gastroesophageal necrosis	2
Gastrocutaneous fistula	1
TOTAL	21

hospital complaining of high fever, nausea, vomiting, and a rigid abdomen. Exploratory laparotomy revealed a defect in the anastomotic area with multiple intra-abdominal abscesses. This patient developed sepsis and multiorgan failure and eventually died. Two of the leaks (15%) were in hand-sewn anastomoses and one (12%) occurred in a stapled anastomosis.

Three patients (14%) developed anastomotic strictures and required endoscopic dilatation. Two of the anastomoses (15%) had been hand sewn and one (12%) was stapled. One of these strictures occurred in a patient with colon interposition. Three patients have since required revision of their esophagojejunostomies, two for stricture and one for reflux esophagitis.

One patient died 4½ months after surgery as a result of septic arthritis, endocarditis, and aspiration pneumonia, and four patients have been lost to follow-up. The other 15 patients were interviewed by phone or in person 1 to 70 months (average 30 months) after completion gastrectomy. They were questioned about their eating habits, weight, gastrointestinal symptoms, and general health.

Three patients (20%) complained of constant or frequent abdominal pain. Three others complained of mild discomfort after eating a large meal. One patient (7%) complained of constant nausea and vomiting and one (7%) complained of constant nausea without vomiting. Four patients reported rare episodes of nausea and vomiting. Four patients (27%) had frequent episodes of diarrhea. Only one patient (7%) complained of bloating and one complained of early satiety (7%) (Fig. 1).

In terms of symptomatic improvement following completion gastrectomy, eight (53%) reported significant improvement, two (13%) reported moderate improvement, and four (27%) reported no improvement in their symptoms. One patient reported worsening of symptoms following the operation, mainly resulting from lack of appetite, abdominal pain, and continued weight loss.

Regarding patients' diets and eating habits, four reported being able to eat normal-sized meals, eight reported eating half their normal amounts, two ate one fourth, and one ate one eighth their normal portions. On average, most ate four to five meals per day with a range of two to eight meals. One patient supplemented his diet with nightly hyperalimentation because of previous small bowel resection and short gut syndrome, and one patient supplemented her diet with Ensure. Five patients reported intolerance to milk, and two could not tolerate lettuce and sweets. Thirteen patients (87%) had a good appetite. Four patients complained of occasional and mild dumping syndrome, which was mostly related to specific foods and was easily relieved.

In terms of objective findings, we calculated the average changes in weight, BWI, albumin, total protein levels, and hematocrit following completion gastrectomy (Table III). There was an average weight loss of

Table III. Objective findings

Parameter	Average change following CG
Weight	2 kg decrease (3%↓*)
Body weight index	0.5 kg/m^2 decrease (2%↓*)
Albumin	0.4 gm/dl decrease (11%↓*)
Total protein	0.3 gm/dl increase (4%↑*)
Hematocrit	1.5% decrease (4%↓*)

CG = Completion gastrectomy
*Not significant by paired *t* test.

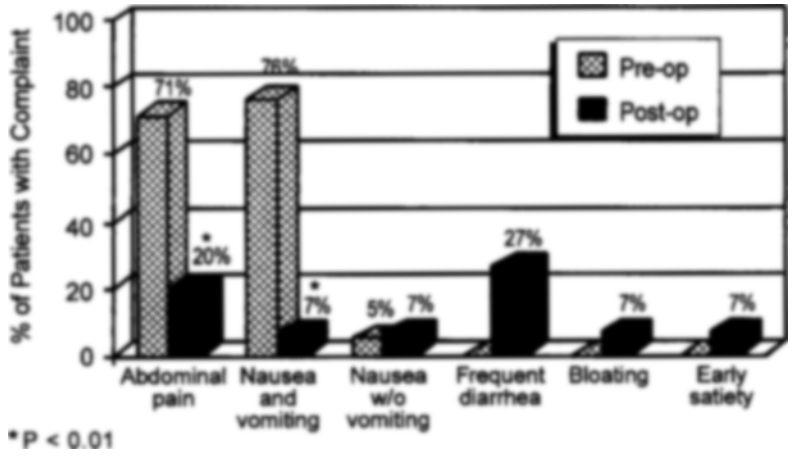

Fig. 1. Symptoms before and after completion gastrectomy. Note that abdominal pain and nausea were significantly reduced, but diarrhea increased.

2 kg, or 3% decrease in body weight. There was a mean decrease of 0.4 gm/dl (range 4.0 to 3.6 gm/dl) in the albumin level and an increase of 0.3 gm/dl (range 6.5 to 6.8 gm/dl) in the total protein level. The hematocrit level showed a mean decrease of 1.5% but despite this small decrease, 60% of our patients had or continue to have mild anemia even though they are being treated with vitamin B_{12} and oral iron supplementation.

Five patients had a BWI of less than 20 kg/m^2 preoperatively and four patients had this same BWI postoperatively (Fig. 2). Two of these patients, although they still had a BWI of less than 20 kg/m^2, in fact had a small increase in BWI and a slight improvement in their nutritional status. There was only one patient with a significant postoperative decrease in BWI to less than 20 kg/m^2.

The percentage changes in objective parameters, including weight, BWI, albumin, total protein, and hematocrit, are shown in Fig. 3, and none of these changes were statistically significant by paired *t* test.

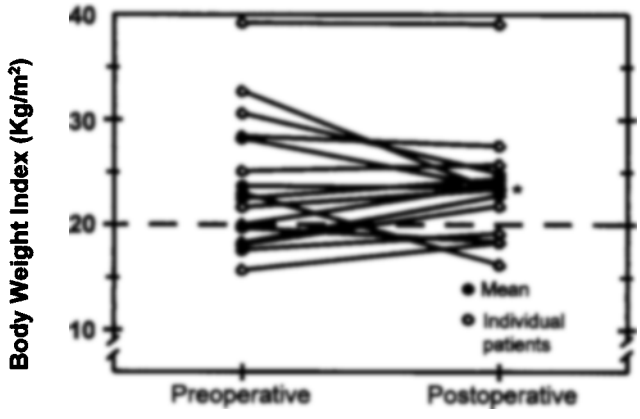

Fig. 2. Changes in body weight index after completion gastrectomy. For the majority of patients there was no change in body weight index.

DISCUSSION

Our patients who were eventually treated with completion gastrectomy and Roux-en-Y esophagojejunostomy for nonmalignant gastric disease had undergone multiple prior gastric operations to relieve their symptoms. Completion gastrectomy was successful in two thirds of these patients. These results are consistent with those of Gustavsson et al.[6,7] from the Mayo Clinic. Also, Eckhauser et al.[8] reported satisfactory results in 86% of their 15 patients who underwent completion gastrectomy for postsurgical gastroparesis syndrome. McCallum et al.[9] examined eight patients with long-term follow-up after completion gastrectomy and found 70% to 90% improvement and satisfaction with clinical results. In one study, two thirds of the patients with chronic gastric atony had improvement of their symptoms after subtotal and near-total Roux-en-Y gastrectomy.[10] Other investigators have reported similar success rates with extensive or near-total Roux-en-Y gastrectomy.[11-14]

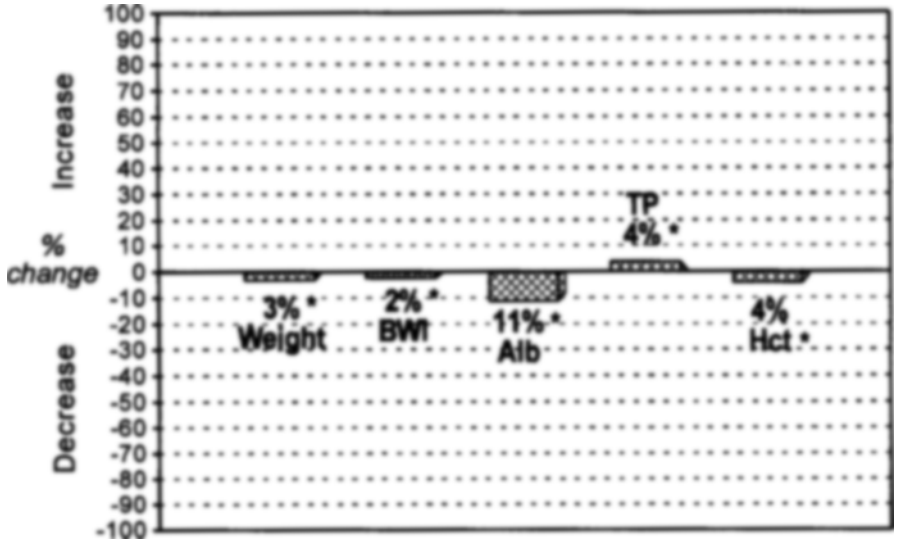

Fig. 3. Changes in markers for malnutrition following completion gastrectomy. There were no significant changes.

Many authors recommend using medical therapy and prokinetic agents to treat patients with chronic gastric atony and Roux stasis syndrome, reserving surgical treatment for those patients in whom dietary restrictions and medical management prove unsuccessful.[15-19] In the majority of our patients, aggressive medical management had failed and, in fact, so too had the more conservative surgical revisions. Our data and those of others support the use of completion gastrectomy when lesser procedures have failed to relieve symptoms.

There are a number of studies dealing with reconstruction of a jejunal reservoir as a gastric substitute after subtotal or total gastrectomy.[20-27] The various forms of pouch reconstruction include the Hunt-Lawrence pouch, the Tanner Roux-19 pouch, the Tolley pouch, and the Lygidakis reconstruction. Lygidakis[27] reported a series of 32 patients who underwent total gastrectomy for benign disease in whom a jejunal pouch was constructed with two jejunojejunostomies. In this study satisfactory results were achieved in 30 of the 32 patients. Others have reported their experience with a reservoir substitute for the stomach after total gastrectomy, yet there is still much debate over whether the presence of a pouch is necessary after total gastrectomy. In our patients we performed an end-to-side Roux-en-Y esophagojejunostomy for the purpose of simplicity and also because there are no specific studies to indicate any benefit of pouch reconstruction after total gastrectomy for benign disease.

In most articles reporting on outcome after completion gastrectomy, long-term follow-up ranges from 1 to 8 years (average of 30 months). In our study the mean follow-up was 30 months.[1-4,7-11] A lengthy follow-up period is important because many of the immediate postgastrectomy symptoms resolve over time and patients learn how to adjust their diets or relieve their minor symptoms. For example, many of the patients in our study have become accustomed to eating three main meals per day with snacks in between. Some modify the size of their meals and are able to tolerate a regular diet with the exception of milk and lettuce. Therefore long-term follow-up and frequent evaluations are crucial to patients who have undergone completion gastrectomy. Braga et al.[28] agree that malnutrition following total gastrectomy can be prevented by adequate calorie intake, and strict nutritional follow-up is essential to ensure adequate food intake. In our study the mortality rate was 5% and morbidity was 24%. These results are consistent with those of other studies.

With the exception of the two patients who required esophagectomy and right colon interposition, the other 19 patients all underwent gastrectomy without any gastric remnant or cuff for the esophagojejunal anastomosis. Esophagojejunostomy can be accomplished with a low incidence of anastomotic leakage. We encountered three cases of anastomotic leakage (14%). Two of these were small contained leaks that were successfully treated by specifying that the patients be given nothing by mouth.

In our study eight of the anastomoses were performed using an EEA stapler (U.S. Surgical Corp., Norwalk, Conn.) and the other 13 were performed in the usual hand-sewn manner, with one leak from the stapled and two from the hand-sewn anastomoses. There were three postoperative anastomotic strictures; two occurred in hand-sewn and one in a stapled anastomosis. The incidence of stricture and leakage in our study was somewhat higher than in previous reports. However, in this small group there was no obvious difference in the incidence of anastomotic leakage or stricture between hand-sewn and stapled anastomoses.

All of our patients underwent a postoperative Gastrografin swallow test to confirm the integrity of the anastomosis. Only one patient had evidence of a defect, which was treated with hyperalimentation and specifying nothing by mouth; repeat studies 1 week later showed no evidence of any anastomotic leakage. Despite normal Gastrografin studies, three of our patients had clinically significant leaks and one, in fact, resulted in death. Therefore a normal Gastrografin study by no means guarantees a perfect anastomosis, and it obviously takes more than 1 week for the inflammation and swelling of the tissue to resolve and an anastomotic defect to become clinically evident. Therefore, the addition of a small rim of stomach to render the anastomosis "leak proof" may, in fact, be quite important, as has been recommended by Miedema and Kelly.[14]

In the Roux-en-Y anastomosis, the Roux limb in all of our patients measured between 40 and 50 cm in length, and two were antecolic, eight were retrocolic, and 11 were not reported. There was no difference in the outcome, although it is often easier to place the Roux limb retrocolic because it allows better closure.

Although total gastrectomy is a radical procedure, it can be performed safely and will provide symptomatic relief in selected patients. Nutritionally, these patients will remain stable if their food intake is adequate. There are many ways to restore gastrointestinal continuity after total gastrectomy; however, the only critical factor seems to be diversion of bile 40 cm from the esophagojejunal anastomosis.

REFERENCES

1. Schrock TR, Way LW. Total gastrectomy. Am J Surg 1978; 135:348-355.
2. Deveney CW, Deveney KE, Way LW. The Zollinger-Ellison syndrome—23 years later. Ann Surg 1978;188:384-393.
3. Deveney CW, Deveney KE, Stark D, Moss A, Stein S, Way L. Resection of gastrinomas. Ann Surg 1983;198:546-553.
4. Thompson JC, Lewis BG, Weiner I, Townsend CM. The role of surgery in the Zollinger-Ellison syndrome. Ann Surg 1983; 197:594-607.
5. Mathias JR, Khanna R, Nealon WH, Browne RM, Reeves-Darby VG, Clench MH. Roux-limb motility after total gastrectomy and Roux-en-Y anastomosis in patients with Zollinger-Ellison syndrome. Dig Dis Sci 1992;37:545-550.
6. Gustavsson S, Ilstrup DM, Morrison P, Kelly KA. Roux-Y stasis syndrome after gastrectomy. Am J Surg 1988;155:490-494.
7. Gustavsson S, Kelly KA. Total gastrectomy for benign disease. Surg Clin North Am 1987;67:539-550.
8. Eckhauser FE, Knol JA, Raper SA, Guice KS. Completion gastrectomy for postsurgical gastroparesis syndrome. Ann Surg 1988;208:345-353.
9. McCallum RW, Polepalle SC, Schirmer B. Completion gastrectomy for refractory gastroparesis following surgery for peptic ulcer disease. Dig Dis Sci 1991;36:1556-1561.
10. Karlstrom L, Kelly KA. Roux-Y gastrectomy for chronic gastric atony. Am J Surg 1989;157:44-49.
11. Vogel SB, Woodward ER. The surgical treatment of chronic gastric atony following Roux-Y diversion for alkaline reflux gastritis. Ann Surg 1989;209:756-762.
12. Hinder RA, Esser J, DeMeester TR. Management of gastric emptying disorders following the Roux-en-Y procedure. Surgery 1988;104:765-772.
13. Sawyers JL. Management of postgastrectomy syndromes. Am J Surg 1990;159:8-14.
14. Miedema BW, Kelly KA. The Roux operation for postgastrectomy syndromes. Am J Surg 1991;161:256-261.
15. Pellegrini CA, Broderick WC, Van Dyke D, Way LW. Diagnosis and treatment of gastric emptying disorders. Am J Surg 1983;145:143-151.
16. Pellegrini CA, Deveney CW, Patti MG, Lewin M, Way LW. Intestinal transit of food after total gastrectomy and Roux-Y esophagojejunostomy. Am J Surg 1986;151:117-125.
17. Karlstrom L, Kelly KA. Ectopic jejunal pacemakers and gastric emptying after Roux gastrectomy: Effect of intestinal pacing. Surgery 1989;106:867-871.
18. Pellegrini CA, Patti MG, Lewin M, Way LW. Alkaline reflux gastritis and the effect of biliary diversion on gastric emptying of solid food. Am J Surg 1985;150:166-171.
19. Woodward A, Schu W, Wojtowycz AR, Sillin LF. Gastric emptying of solids after Roux-en-Y gastrectomy: Is extragastric vagal innervation important? Surgery 1991;110:793-798.
20. Mathias JR, Fernandez A, Sninsky CA, Clench MH, Davis RH. Nausea, vomiting and abdominal pain after Roux-en-Y anastomosis: Motility of the jejunal limb. Gastroenterology 1985;88:101-107.
21. Vogel SB, Hocking MP, Woodward ER. Clinical and radionuclide evaluation of Roux-Y diversion for postgastrectomy dumping. Am J Surg 1988;155:57-62.
22. Delcore R, Cheung LY. Surgical options in postgastrectomy syndromes. Surg Clin North Am 1991;71:57-75.
23. Eagon JC, Miedema BW, Kelly KA. Postgastrectomy syndromes. Surg Clin North Am 1992;72:445-465.
24. Behrns KE, Sarr MG. Diagnosis and management of gastric emptying disorders. Adv Surg 1994;27:233-255.
25. Summers GE Jr, Hocking MP. Preoperative and postoperative motility disorders of the stomach. Surg Clin North Am 1992;72:467-485.
26. Camilleri M. Appraisal of medium- and long-term treatment of gastroparesis and chronic intestinal dysmotility. Am J Gastroenterol 1994;89:1769-1774.
27. Lygidakis NJ. Long term results of a new method of reconstruction for continuity of the alimentary tract after total gastrectomy. Surg Gynecol Obstet 1984;158:335-338.
28. Braga M, Zuliani W, Foppa L, DiCarlo V, Cristallo M. Food intake and nutritional status after total gastrectomy: Results of a nutritional follow-up. Br J Surg 1988;75:477-480.

Intra-Abdominal Abscesses Following Laparoscopic and Open Appendectomies

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Recent findings in a small number of studies have suggested a trend toward increased infectious complications following laparoscopic appendectomy. The purpose of the present review was to evaluate the incidence of postappendectomy intra-abdominal abscess formation following laparoscopic and open appendectomies. Using the surgical database of the Los Angeles County–University of Southern California Medical Center, we reviewed the records of all appendectomies performed at the center between March 1993 and September 1995. Incidental appendectomies as well as appendectomies in pediatric patients under the age of 18 years were excluded. A total of 2497 appendectomies were identified; indications for these procedures included acute appendicitis in 1422 cases (57%), gangrenous appendicitis in 289 (12%), and perforated appendicitis in 786 (31%). The intraoperative diagnosis made by the surgeon was used for classification. A two-tailed P value of <0.05 was considered significant. There was no significant difference in the rate of abscess formation between the groups undergoing open and laparoscopic appendectomies for acute and gangrenous appendicitis. In patients with perforated appendicitis, a total of 26 postappendectomy intra-abdominal abscesses occurred following 786 appendectomies for an overall abscess formation rate of 3.3%. Eighteen abscesses occurred following 683 open appendectomies (2.6%), six abscesses occurred following 67 laparoscopic appendectomies (9.0%), and the remaining two abscesses occurred following 36 converted cases (5.6%). For perforated appendicitis, however, there was a statistically significant increase in the rate of abscess formation following laparoscopic appendectomy compared to conventional open appendectomy (9.0% vs. 2.6%, $P = 0.015$). There was no significant difference in the rate of abscess formation between open vs. converted cases or between laparoscopic vs. converted cases. A comparison of the length of the postoperative hospital stay showed no significant difference between open and laparoscopic appendectomy for perforated appendicitis (6.1 days vs. 5.9 days). Laparoscopic appendectomy for perforated appendicitis is associated with a higher rate of postoperative intra-abdominal abscess formation without the benefit of a shortened hospital stay. Given these findings, laparoscopic appendectomy is not recommended in patients with perforated appendicitis. (J GASTROINTEST SURG 1997;188-193.)

Since its introduction by Semm[1] in 1982, laparoscopic appendectomy has become a common procedure in the treatment of appendicitis. Many investigators have reported numerous advantages of the laparoscopic technique over the conventional open technique. Most of these published reports, however, have provided little conclusive data on the complications of appendicitis.[2-5]

Recently, a small number of studies have been conducted to define the efficacy of laparoscopic appendectomy in the treatment of perforated appendicitis. Data from these studies have suggested a trend toward increased infectious complications following the laparoscopic procedure for perforated appendicitis compared to the traditional open procedure. However, most of these studies have dealt with a relatively small number of cases.[6-8] Our current study retrospectively examines a total of 2497 appendectomies performed at the Los Angeles County–University of Southern California (LAC-USC) Medical Center. The purpose of this study was to evaluate the incidence of intra-abdominal abscess formation fol-

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lowing laparoscopic and conventional open appendectomies.

MATERIAL AND METHODS

The computerized database of the LAC-USC hospital was searched for all patients who had undergone appendectomies over the 3½-year study period, which lasted from March 1992 to September 1995. Incidental appendectomies as well as appendectomies in pediatric patients under the age of 18 years were excluded from this study. Current procedural codes and international current diagnosis codes were used for the database search. The intraoperative diagnosis made by the surgeon was used for classification. Postoperative hospital stay was calculated using the dates of operation and discharge. Information regarding sex, race, and age at the time of surgery was also obtained. The charts of all patients who developed intra-abdominal abscesses were reviewed. CT reports from those patients were also reviewed to confirm the diagnosis and to classify the location of the abscesses.

Laparoscopic appendectomies were performed using three trocars. A pneumoperitoneum was established after placement of a 10 mm trocar in the infraumbilical position by means of the Hasson technique. The abdomen was then explored using a 10 mm 30-degree laparoscope. After the diagnosis was confirmed, an additional 10 mm trocar was placed in the right upper quadrant. The appendix was then mobilized and grasped with endoscopic Babcock-type forceps. The mesoappendix was then either serially clipped or divided using an endoscopic linear stapler that was placed via a trocar in the left lower quadrant. The base of the appendix was either double ligated with pretied ligatures or, more commonly, divided with an endoscopic linear stapler. The appendix was then either removed through a trocar or placed in a bag and removed through the wound. After removal of the appendix, the staple line was visually inspected. The pelvis and the right lower quadrant were then irrigated and suctioned dry. Drains were not commonly used. Antibiotic treatment of acute appendicitis consisted of a single broad-spectrum cephalosporin, usually cefoxitin, which was continued for 24 hours. Antibiotic therapy for perforated appendicitis generally consisted of a combination of aztreonam and metronidazole. Antibiotics were normally continued for 5 days and were discontinued if the patient had been afebrile for at least 48 hours.

Statistical analysis was carried out by means of Fisher's exact test using an SAS program by the biostatistics division of the University of Southern California. Two-tailed P values <0.05 were considered significant.

RESULTS

A total of 2497 appendectomies were performed over the 3½-year study period with an average of two operations per day. Fifty-seven percent (n = 1422) of the procedures were performed for acute appendicitis, 12% (n = 289) for gangrenous appendicitis, and the remaining 31% (n = 786) for perforated appendicitis. Of the 2497 appendectomies, 81% (n = 2034) were performed using the conventional open technique and 17% (n = 413) were completed laparoscopically. In 2% (n = 50) of the cases, technical difficulties necessitated conversion from laparoscopic to open appendectomy.

Of the open appendectomies (n = 2034), 55% were for acute appendicitis, 11% were for gangrenous appendicitis, and the remaining 34% were for perforated appendicitis. Of the laparoscopic appendectomies (n = 413), 69% were for acute appendicitis, 15% were for gangrenous appendicitis, and the remaining 16% were for perforated appendicitis. Thirty-four percent of all open appendectomies vs. only 16% of all laparoscopic appendectomies were for perforated appendicitis. The age distributions of patients in the open appendectomy and the laparoscopic appendectomy groups were almost identical (Table I).

Among patients with acute appendicitis, a total of six abscesses occurred following 1422 appendectomies yielding an overall abscess formation rate of 0.4%. Three abscesses occurred following open appendec-

Table I. Age distributions for various procedures

Procedure	Age (yr)					
	18-20	21-30	31-40	41-50	51-60	>60
Open (%)	10	50	25	9	4	2
Laparoscopic (%)	12	50	25	10	3	1
Laparoscopic-open (%)	6	42	26	12	8	6

Table II. Abscess formation following appendectomy

	No. of patients	No. of abscesses	*P* value
Acute appendicitis	1422		0.1 (NS)
Laparoscopic	285	3 (1.1%)	
Laparoscopic-open	10	0	
Open	1127	3 (0.3%)	
Gangrenous appendicitis	289		0.4 (NS)
Laparoscopic	61	1 (1.6%)	
Laparoscopic-open	4	0	
Open	224	1 (0.5%)	
Perforated appendicitis	786		0.015
Laparoscopic	67	6 (9.0%)	
Laparoscopic-open	36	2 (5.6%)	
Open	683	18 (2.6%)	

NS = not significant.

Table III. Intra-abdominal abscess location

Area	No.
Right upper quadrant	1
Left upper quadrant	0
Midabdomen	3
Right lower quadrant	29
Left lower quadrant	3
Pelvis	11

tomy and the other three occurred following laparoscopic appendectomy. There was no significant difference in the rate of abscess formation between the groups undergoing open vs. laparoscopic appendectomy for acute appendicitis. In patients with gangrenous appendicitis, two abscesses occurred following appendectomy in 289 cases for an overall abscess formation rate of 0.7%. One abscess occurred following open appendectomy and the other abscess following laparoscopic appendectomy. Again, for gangrenous appendicitis, there was no significant difference in the abscess formation rate between the open and laparoscopic procedures.

Among patients with perforated appendicitis, a total of 26 intra-abdominal abscesses occurred following appendectomy in 786 cases for an overall abscess formation rate of 3.3%. Eighteen abscesses occurred following conventional open appendectomy, six abscesses occurred following laparoscopic appendectomy, and the remaining two abscesses occurred following conversion from laparoscopic to open appendectomy. In the group with perforated appendicitis, however, there was a statistically significant increase in the rate of intra-abdominal abscess formation following laparoscopic appendectomy compared to open appendectomy (9.0% vs. 2.6%, $P = 0.015$) (Table II).

Concerning the location of the intra-abdominal abscesses, 29 abscesses were found in the right lower quadrant, 11 were found in the pelvic area, and three were found in the midabdominal area; another three abscesses were found in the left lower quadrant. Only one abscess was found in the right upper quadrant and no abscesses were found in the left upper quadrant (Table III).

A comparison of average hospital stays showed no significant difference between conventional open and laparoscopic appendectomy for perforated appendicitis (6.1 days vs. 5.9 days) or for gangrenous appendicitis (4.5 days vs. 4.6 days). For acute appendicitis, however, there was a significant difference in the average hospital stay between open and laparoscopic procedures (3.1 days vs. 2.2 days, $P = 0.0001$) (Table IV). There was no difference in the rate of abscess formation between males and females among patients with perforated (3.3% vs. 3.3%) or acute and gangrenous appendicitis as a group (0.5% vs. 0.4%).

DISCUSSION

Since the introduction of laparoscopic appendectomy, numerous advantages of this technique have been reported. These include a shorter hospital stay, earlier return to normal activity, less pain, and improved cosmesis. However, as more experience has been gained, there has been increasing concern regarding the efficacy of this new procedure in cases of complicated appendicitis, especially perforated ap-

Table IV. Length of hospital stay (days)

	Procedure			
Appendicitis	Open	Laparoscopic-open	Laparoscopic	*P* value
Acute	3.1 ± 3.0	3.2 ± 2.1	2.2 ± 2.0	<0.0001
Gangrenous	4.5 ± 2.2	4.3 ± 1.8	4.6 ± 3.2	NS
Perforated	6.1 ± 3.9	6.0 ± 2.3	5.9 ± 2.7	NS

All values are mean ± standard deviation; NS = not significant.

pendicitis. A trend toward more postoperative infectious complications has been noted in a few studies, but the small number of cases failed to provide adequate capability to detect statistically significant differences. Our current study included a total of 2497 appendectomies, the largest study population assembled to date to report on this subject.

Postoperative intra-abdominal abscess formation following appendectomy is a serious disease state. Intraperitoneal abscess with consequent sepsis is responsible for almost all reported deaths following appendectomy. For this reason postoperative intraabdominal abscess formation seems to be a good indicator of the likelihood of postappendectomy complications.

For acute or gangrenous appendicitis, our study found no difference in the rate of intra-abdominal abscess formation between patients undergoing conventional open vs. laparoscopic procedures. In addition, the length of hospital stay was significantly decreased in patients with acute appendicitis following laparoscopic appendectomy as compared to open appendectomy. The laparoscopic technique appears to be safe and effective for use in cases of acute and gangrenous appendicitis. These findings are consistent with those of previous reports. Many reports have also suggested that laparoscopy allows surgeons to thoroughly evaluate not only the appendix but also the entire abdomen. Therefore laparoscopy seems to be a useful diagnostic tool for patients with equivocal findings, especially women and those in whom the diagnosis of appendicitis is questionable. Some investigators have even suggested that peritoneal lavage may be much more complete and effective when the laparoscopic procedure is used.

In this study, however, laparoscopic appendectomy for perforated appendicitis was associated with a higher rate of intra-abdominal abscess formation compared to the conventional open procedure (9.0% vs. 2.6%, $P = 0.015$). Subset analysis of patients with perforated appendicitis in previous studies suggested a similar finding, that is, an increased tendency toward more infectious complications following laparoscopic appendectomy. However, the reason for the increased rate of intra-abdominal abscess formation following laparoscopic appendectomy for perforated appendicitis is not clear. Some researchers have suggested that the pneumoperitoneum may possibly prompt conversion of a local abscess or inflammatory reaction to a diffuse abdominal process by inadvertently seeding the entire abdomen. Others have suggested that perhaps the carbon dioxide used for peritoneal insufflation promotes anaerobic bacterial growth following the laparoscopic approach. However, there are no published data to support these speculations. In addition, the fact that most intra-abdominal abscesses were found in the right lower quadrant and pelvic area does not support the mechanism of possible diffusion. More studies must be conducted to clarify this matter.

In addition to its association with a higher rate of intra-abdominal abscess formation, laparoscopic appendectomy for treatment of perforated appendicitis did not provide the benefit of a shortened hospital stay compared to conventional open appendectomy (5.9 vs. 6.1 days). Oftentimes patients with intra-abdominal abscesses will require additional procedures and prolonged intravenous antibiotic treatment. The increased rate of abscess formation without the benefit of a shorter hospital stay negates the primary benefits of the laparoscopic procedure in cases of perforated appendicitis.

CONCLUSION

Our study shows no significant difference in the rate of postoperative intra-abdominal abscess formation between laparoscopic and open appendectomies in cases of acute or gangrenous appendicitis. For perforated appendicitis, however, there is a statistically significant difference in the rate of postoperative intra-abdominal abscess formation between laparoscopic appendectomy and conventional open appendectomy. Laparoscopic appendectomy for perforated

appendicitis is associated with a higher rate of postoperative intra-abdominal abscess formation without the benefit of a shortened hospital stay as compared to open appendectomy. Given these findings, laparoscopic appendectomy is not recommended in patients with perforated appendicitis.

REFERENCES

1. Semm K. Endoscopic appendectomy. Endoscopy 1993;15:59-61.
2. Attwood SEA, Hill ADK. A prospective randomized trial of laparoscopic versus open appendectomy. Surgery 1992;112: 497-501.
3. Ortega AE, Hunter JG. A prospective, randomized comparison of laparoscopic appendectomy with open appendectomy. Am J Surg 1995;169:208-211.
4. Buckley RC, Hall TJ. Laparoscopic appendectomy: Is it worth it? Am Surg 1994;60:30-34.
5. Vallina VL, Velasco JM, McCulloch CS. Laparoscopic versus conventional appendectomy. Ann Surg 1993;218:685-692.
6. Bonanni F, Reed J III. Laparoscopic and conventional open appendectomy. J Am Coll Surg 1994;179:273-278.
7. Martin LC, Puente I. Open versus laparoscopic appendectomy. Ann Surg 1995;222:256-262.
8. Franzee RC, Bohannon WT. Laparoscopic appendectomy for complicated appendicitis. Arch Surg 1996;131:509-512.

Discussion

Dr. L. Way (San Francisco, Calif.). Why do you suppose the incidence of abscess formation is greater in those with perforated appendicitis when they are treated laparoscopically as compared to open appendectomy? What are you doing in the open cases that you are not doing in the laparoscopic ones? Can you change the laparoscopic technique and improve the results?

Dr. G.J. Anthone. That is the question we would all like to answer. Allow me to speculate. In treating a fairly large number of cases of perforated appendicitis laparoscopically, I just want to stick my hand down into that right lower quadrant and break up those inflammatory adhesions and most likely drain that area of infection. I do not think that can be done laparoscopically. Another reason for abscess formation might be that the entire appendix is not removed at the time of a laparoscopic appendectomy. I think that in cases of perforated appendicitis, the actual base of that appendix is difficult to identify. We have actually had some patients referred for reoperation in whom this has occurred.

Dr. N. Soper (St. Louis, Mo.). Do you therefore recommend that an imaging study be performed preoperatively to identify those patients who have a ruptured appendix and thus avoid a laparoscopic approach? Do you recommend, if the appendix is found to be perforated at the time of laparoscopic exploration, immediate conversion to an open operation? I have intuitively thought that in cases of perforation one could actually perform a more satisfactory and complete irrigation of the field laparoscopically than with an open approach. I wonder what maneuvers you perform intraoperatively to prevent these postoperative problems.

Dr. Anthone. I believe irrigation is of very little benefit, although it can be easily accomplished via the laparoscopic technique.

We would like to determine which patients would not benefit from a laparoscopic appendectomy. We would like to say a patient with a temperature of 101.5° F and a white blood cell count greater than 20,000/mm^3, but in order to carry out those studies we would need to include approximately 300 patients in the laparoscopic perforated group, and I do not think we will ever reach that goal. Currently, if a patient has had symptoms for more than 48 hours, has diffuse peritonitis, or has a palpable mass in the right lower quadrant, we will not begin the operation laparoscopically.

Dr. F.C. Brunicardi (Houston, Tex.). Do you use an Endo loop or Endo GIA stapler to remove the appendix? Have you actually analyzed your data to determine whether you can predict which patients have suffered a perforation and which ones have not, and therefore avoid the laparoscopic appendectomy.

Dr. Anthone. To accumulate sufficient data for analysis to determine which patients would not benefit, or which patients have suffered a perforation, we would have to include approximately 300 patients in the laparoscopic perforated group. We have 67, which is far and away the largest of any series published to date. Knowing what we know now, I do not think we will ever reach that 300 mark. So I do not think we will be able to answer that question.

Our technique is now fairly standard. We use a Hasson technique to enter the abdomen, we next place a 5 or 10 mm trocar in the right upper quadrant to grasp the appendix, and a 10 or 12 mm trocar is then inserted in the left lower quadrant for placement of the Endo GIA stapler. We have abandoned the use of Endo loops. We were part of a prospective randomized study early on in 1992, and since the conclusion of that study we have stopped using Endo loops.

Dr. S. Melvin (Columbus, Ohio). Based on six postoperative infections, you have made fairly broad recommendations that laparoscopy should not be used in cases of perforated appendicitis. Did those six infections occur early on or were they noted later in your series? I would agree with your comments that it is considered a technical failure not to break up a loculated abscess, or certainly to leave part of the appendix, and that should be an indication for conversion to an open procedure. Additionally, have you adopted any techniques such as early containment of the appendix in

a plastic bag or other extraction device to avoid "dragging" the infected appendix throughout the abdominal cavity?

Dr. Anthone. The same number of abscesses developed later on in the study as occurred during the early period. We even broke the study period up into 6-month blocks when each group of new residents arrives, and we found no differences.

Regarding removal of the appendix, it was always removed either inside the trocar or in a sterile bag.

Dr. J. Hunter (Atlanta, Ga.). In the case of a free perforation without loculations, if the area is thoroughly irrigated after the appendix is removed, that is probably sufficient. But I think the patients you are referring to are those with more advanced delayed presentations with dangerous abscesses, which I think all of us would agree should be converted to an open procedure.

Dr. Anthone. Our threshold for conversion to an open procedure in that type of a case is very low. Once we look down into the right lower quadrant and see that the appendix cannot be grasped or there is a large phlegmon, the patient is opened.

Dr. F. Quijano (Mexico City, Mexico). Did you compare the hospital costs for laparoscopic vs. open appendectomy?

Dr. Anthone. In this particular study we did not. In the study Dr. Hunter referred to, there was more cost involved in the laparoscopic group. We had a 35% conversion rate for perforated appendicitis, which has got to be expensive, and I do not think the cost is worth it.

Ectopic Expression of *reg* Protein: A Marker of Colorectal Mucosa at Risk for Neoplasia

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Pancreatic regenerating gene *(reg I)* messenger RNA is overexpressed within the pancreas following injury and resection. Its level of expression corresponds to the level of cellular dedifferentiation. Human *reg I* has been localized to chromosome 2p12, and ectopic expression of its mRNA has been found within colorectal tumors. We postulated that colorectal production of *reg I* might either be a marker for the presence of cancer or define mucosa at risk for development of neoplasia. Using a monoclonal antibody to *reg I*, regenerating gene protein was histochemically mapped in 56 cases of documented colorectal adenocarcinoma. In sections of colon from normal control subjects no *reg I* protein was noted, whereas 58.9% of the specimens from cancer patients stained positive for *reg I*. Although a correlation was noted between *reg I* staining and Dukes' stage, there was no correlation with histologic grade or 5-year patient survival. In 39 of 55 cancer specimens the transition zone (interface) between the neoplasm and normal mucosa was visualized; 100% of the transition zones contained cells that stained strongly positive for *reg I*. We conclude that *reg I* protein is ectopically expressed in colorectal mucosa at the transition zone of colorectal cancer, and occasionally within the tumor itself. Although ectopic *reg I* expression in colorectal epithelia is not a marker for the presence of carcinoma, it may be a sensitive marker for mucosa at risk for development of neoplasia. (J GASTROINTEST SURG 1997;194-202.)

The pancreatic regenerating gene *(reg I)* and its protein product are expressed within the exocrine cells of the pancreas.[1,2] *reg I* is part of a family of at least three proteins, all of which have structural homology to calcium-dependent lectins.[2] The messenger RNA sequence of rat *reg I* was originally described by Terazono et al.[1] The genomic sequence is found in humans as well,[3] and the deduced amino acid sequence is identical to that of pancreatic stone protein and pancreatic thread protein.[2] *reg I* mRNA is overexpressed within pancreatic cells following pancreatitis or resection, and its protein product is believed to be involved in the regeneration of islets from ductular precursor cells.[1,2]

Human *reg I* protein is composed of 166 amino acids and ranges from 13 to 18 kd in size, depending on the level of glycosylation.[4] The *reg I* gene exists as a single copy gene that spans approximately 3.0 kilobase pairs and is composed of six exons and five introns. Its TATA box and CCAAT boxlike sequences are located at 27 and 100 base pairs upstream from the transcriptional initiation site.[3] We have recently mapped human *reg I* to chromosome 2p12.[5]

Factors that control production of this acinar cell product are, to date, unknown. It is constituitively expressed in pancreatic acinar cells and much less so in gastric mucosa and renal parenchyma.[3] Inasmuch as it was induced in regenerating pancreatic tissue following resection or pancreatitis, Rouquier et al.[6] proposed that *reg I* is induced within pancreatic cells during the dedifferentiated state. We recently demonstrated that in the cell line AR42J, levels of *reg I* mRNA and protein inversely correlate with the level of cellular differentiation.[7] Watanabe et al.[3] found ectopically expressed *reg I* mRNA in some colorectal tumors, which suggested that *reg I* gene expression was related to tumorigenesis.

Since *reg I* expression appears to be linked to the

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development of colorectal tumors, a qualitative study to localize ectopic production of pancreatic *reg I* protein in colorectal tumors was undertaken. Using a monoclonal antibody to human *reg I*, immunohistochemical analysis of colorectal tumors was performed. The reactivity of the tissues with antihuman *reg I* antibody was visualized and the pattern was evaluated.

MATERIAL AND METHODS

Fifty-six cases of colonic adenocarcinoma were selected from the tumor registry of Montefiore Hospital. The patients were diagnosed with this cancer between 1988 and 1990 and were followed for 5 years after the diagnosis. The group included 26 men and 30 women. The mean age was 77 years. The site of the cancer was the right colon in 26 cases (48%), the transverse colon in three (6%), the descending colon in five (9%), and the rectosigmoid colon in 20 (37%). All patients underwent surgical resection, and based on the final pathology report the caner was classified as Dukes' stage A in 14.8%, stage B in 38.8%, stage C in 27.7%, and stage D in 18.5%. At the end of 5 years, 43% of the patients were still alive.

Sections of normal colon were obtained from three patients undergoing resection for benign disease, and specimens of pancreata were obtained from six additional patients undergoing resection for chronic pancreatitis. These samples were fixed and embedded in paraffin and served as negative and positive controls, respectively.

The formalin-fixed, paraffin-embedded blocks were sliced into sections 5 μm thick using a microtome. Tissue section slides were dewaxed in iodine/xylene solution and rehydrated through graded alcohol series and water. After rinsing the slides in distilled water, the endogenous peroxidase activity was quenched by incubating them in 0.3% H_2O_2/methanol for 30 minutes at 75° C. After a 5-minute equilibration period in phosphate-buffered saline solution (PBS) (pH 7.4), the sections were treated with pepsin (0.01%, Sigma Chemical, St. Louis, Mo.) and incubated at room temperature for 30 minutes. They were then treated for 20 minutes with normal blocking serum (ABC kit, Vector Laboratories, Inc., Burlingame, Calif.). Overnight incubation at room temperature was followed by the addition of monoclonal antibody D4 (raised to human pancreatic stone protein) (Immunotech, Inc., Westbrook, Maine)[8] at a 1:4000 dilution. After rinsing with PBS, slides were incubated with biotinylated antimouse immunoglobulin G and (Vectastain Elite ABC reagent, Vector Laboratories, Inc.) for 30 minutes. The sites of bound antibody were visualized by incubation in 3,3'-diaminobenzidine tetrahydrochloride-H_2O_2 substrate solution. The sections were then rinsed in deionized water, counterstained with hematoxylin, dehydrated, and mounted.

Molecular Analysis of Colon Tissue

Total cellular protein and RNA were isolated from samples of mucosa at the normal-to-cancer transition zone from two patients by means of the TRI-Reagent technique (Molecular Research Corp., Cincinnati, Ohio); 10 ug of protein was subjected to Western blot analysis using monoclonal antibody D4, and 10 ug of RNA was subjected to Northern blot analysis using the antisense probe to rat *reg I* as previously described.[9]

Statistical Analysis

Data were entered in tabular form into a computer database for analysis (Statistica, Statsoft, Tulsa, Okla.) and subjected to nonparametric analysis. A P value <0.05 was considered significant.

RESULTS

The control sections (from the six pancreatectomy and the three colectomy patients) stained positive and negative for the antibody, respectively (Fig. 1).

Histologically, the 56 tissue sections analyzed exhibited three types of tissue: (1) colonic tissue bordering the malignancy, which included normal and cancerous tissue; (2) malignancy only, with no identifiable normal colonic mucosa; and (3) adenomatous polyps containing varying foci of malignancy.

Immunohistochemistry

Normal Colon-to-Colon Cancer Interface. Thirty-nine of the 56 sections contained normal colonic mucosa bordering malignancies of varying histologic grades and stages of invasion. In all 39 sections, a positive reaction with the *reg I* antibody was seen in the colonic epithelium adjacent to neoplastic tissue, manifested as diffuse dark granules within the cytoplasm of the crypt cells (both goblet and epithelial cells) (Fig. 2, *A* and *B*). *reg I* density was semiquantified by estimating the number of positive cells. Greatest *reg I* positivity was typically noted in crypts located within 1 mm of the adjacent malignancy. Histologically this region correlated with the junction between the normal epithelium and the malignancy. This epithelium was nonneoplastic and marked occasionally by hypertrophied, branched glands. With increasing distance

Fig. 1. Control specimens of pancreas **(A)** and colon **(B)** stained with monoclonal antibody to *reg I*. The pancreas is from a patient who underwent pancreatectomy for chronic pancreatitis. All of the acinar cells stained positive, whereas no islets stained (not shown). Positive cells contain dark granules within the cytoplasm. No cells in the colonic mucosa from a normal colon resected for diverticulitis stained positive for *reg I* (×40).

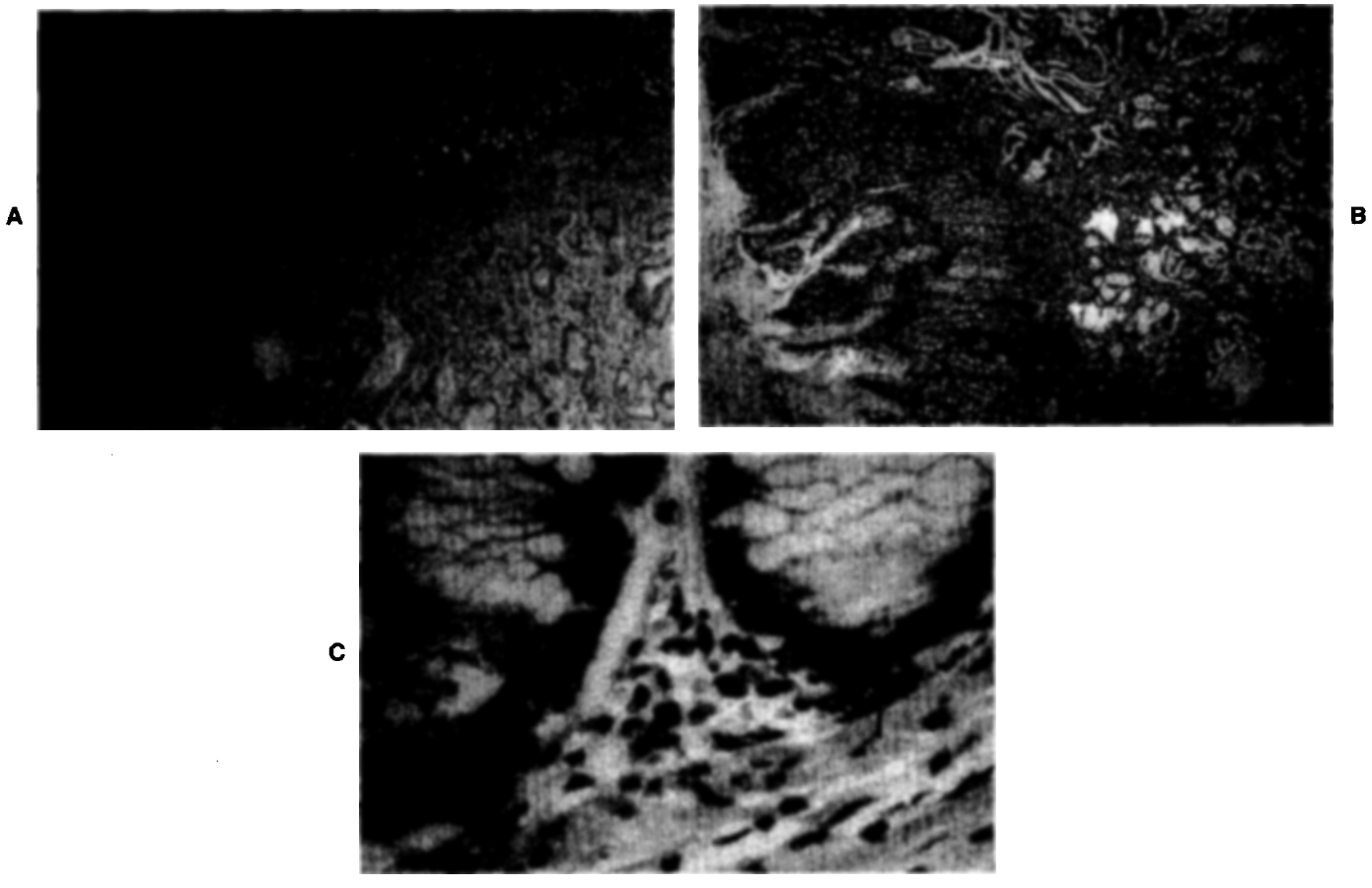

Fig. 2. A and **B,** Specimens from two different patients with depiction of the normal colon-to-colon cancer interface. In both *A* and *B* the normal-appearing mucosa at the interface with the cancer stained positive for *reg I*, and staining was concentrated within the crypts (×200). The tumor in *A* (lower right portion of the panel) was not positive for *reg I*, whereas the tumor in *B* (right half of the photomicrograph) was positive for *reg I*. **C,** High-power view of a single positive cell *(arrow)* at the base of an otherwise normal crypt 1 cm from the interface of the tumor seen in *A*.

away from the tumor border in the direction of the normal epithelium, the positivity decreased markedly. However, minute (or isolated) yet detectable positivity was seen as far as 1 cm away from the malignancy, in crypts manifesting no other histologic abnormality (Fig. 2, *C*).

The location of *reg I* protein within the crypts also varied with the crypt's distance from the malignancy. In normal crypts located far from the malignancy, *reg I* protein was observed in a few cells at the base of the crypts. Closer to the malignancy more cells reacted with the antibody, and positivity was seen to move upward from the bottom toward the neck of the crypt. At the point of greatest positivity, within 1 mm from the malignancy, the entire crypt usually stained.

In 22 of the 39 sections previously described, the bordering malignancies also reacted with the anti-*reg I* antibody. The positivity appeared as dark cytoplasmic granules within the cells of the malignant glands. The degree of positivity seemed to correlate with the degree of differentiation of the malignancy; the more well-differentiated glands stained more densely for *reg I* (see below).

Colon Cancer Alone. Nine of the sections studied contained only tumor. The pattern of *reg I*-positive reaction was similar to that seen in the malignancies described in the preceding section—that is, the well-differentiated glands reacted with the antibody (n = 3) whereas the more poorly differentiated glands did not (n = 6). Of the 48 frank cancers studied (in this and the preceding section), 25 (52.1%) stained positive for *reg I*.

Malignant Polyps. Eight specimens were of adenomatous polyps (obtained from polypectomy) harboring malignancies ranging from a focal point of carcinoma to a point where the adenoma was extensively replaced by cancerous cells. Five villous and three tubulovillous adenomas constituted this group. *reg I* positivity was seen in all eight adenomas, which were localized to a few adenomatous glands in close proximity to the carcinomatous foci (Fig. 3). All eight polyps contained well-differentiated malignancies and these malignant glands were all positive for *reg I* in varying degrees. Localization of *reg I* protein in the normal mucosa adjacent to the polyps was difficult to evaluate because of its unavailability in polypectomy specimens.

Molecular Analysis

Western blot analysis of protein extracted from transition zones of two patients revealed a band at 14 to 18 kd, consistent with human *reg I* (Fig. 4). Northern blot analysis revealed a single band at 0.9 kilobase identical to the region where *reg I* is found (data not shown). The intensity of the band was less than one tenth that of pancreatic tissue (similar to that described by Watanabe et al.[3]). The surrounding normal tissue had no such reactivity.

Statistical Analysis

As mentioned previously, 100% of the transition zones between cancer and normal mucosa were positive for *reg I*. Of all the cancerous tissue studied, 33 specimens (58.9%) stained positive for *reg I*. No correlation between positivity and location of tumor in the colon was noted. Specifically, half of the 26 right colon tumors were positive for *reg I*, as was one of the three transverse colon tumors. Thirteen of the 20 rec-

Fig. 3. Photomicrograph of an adenomatous polyp. Adenomatous glands within the polyp stained positive for *reg I*, as did the carcinoma arising from within the polyp (middle-lower portion of the photomicrograph).

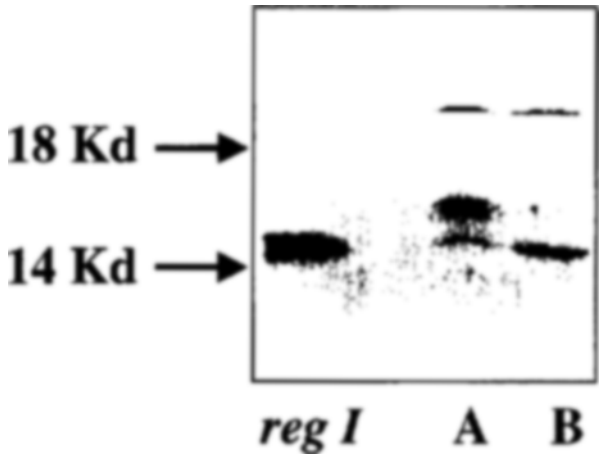

Fig. 4. Western blot analysis of protein isolated from the transition zones from two colorectal tumors (*A* and *B*) using *reg I* monoclonal antibody. The control specimen is human *reg I* isolated from human pancreas as described in previous reports.[13,14] The variable size of *reg I* is likely due to differences in glycosylation. Protein isolated from mucosa 5 cm from the lesion was negative for *reg I* (not shown).

tosigmoid tumors and all of the five descending colon tumors were positive, indicating a slight predilection for tumors of the left side to express the protein.

When we compared *reg I* expression within the cancer and histologic differentiation of the tumor, no significant correlation was noted ($P = 0.12$, Spearman's rank correlation coefficient). Of the *reg I*–positive tumors, 16 were well differentiated, 11 were moderatedly differentiated, and five were poorly differentiated. Of the *reg I*–negative tumors, the distribution was 6, 11, and 5, respectively. This tended toward statistical significance when the tumors were grouped according to whether they were well differentiated vs. moderately to poorly differentiated ($P = 0.08$ Fisher's exact test). Specifically, 16 well-differentiated and 16 moderately to poorly differentiated tumors were *reg I* positive, whereas 6 well-differentiated and 16 moderately to poorly differentiated tumors were *reg I* negative.

A significant correlation was noted between Dukes' classification and tumor expression of *reg I* ($P = 0.01$, Spearman's rank correlation coefficient). This is likely due to the fact that all eight Dukes' A tumors were positive for *reg I*, whereas 9 of 21 Dukes' B, 6 of 12 Dukes' C, and 8 of 12 Dukes' D tumors were positive. When we grouped tumors into Dukes' A vs. Dukes' B-D tumors and compared *reg I* gene expression in these tumors, a significant correlation was noted. Specifically, the ratio of Dukes' A/Dukes' B-D tumors was 8 of 23 for the *reg I*–positive tumors vs. 0 of 22 *reg I*–negative tumors ($P = 0.008$, Fisher's exact test).

No statistical correlation was noted between the presence of *reg I* and 5-year survival.

DISCUSSION

The pancreatic regenerating gene family has been identified as pancreatic acinar cell products in humans, rats, and mice.[1-3,10] The sequence of the three protein products of the *reg* family predicts them to be calcium-dependent lectins[2] but their function is unknown. The amino acid sequence of human *reg I* has been shown to be identical to that of pancreatic stone protein (PSP, lithostatine), which is found in the stones of patients with chronic calcific pancreatitis,[11,12] and pancreatic thread protein,[13,14] a secretory protein that polymerizes in vitro under specific conditions. The amino acid sequence of *reg III* is identical to pancreatitis-associated protein (PAP), which is a protein induced and released into the serum during acute pancreatitis,[15-17] and HIP, a protein expressed within hepatocellular carcinomas (named for its expression in *h*epatoma, small *i*ntestine, and *p*ancreas).[18] The nature of *reg II* is currently unknown. The genes of human *reg I* and *reg III* have both been localized to chromosome 2p12.[5,19]

reg I mRNA is normally expressed in pancreatic acinar tissue but not in ductular cells or in mature islets.[1] It is induced during islet regeneration following pancreatectomy and during islet hyperplasia.[9,20] *reg I* protein is mitogenic to pancreatic ductular and β-cells but not acinar cells or islets.[21,22] These studies suggest that *reg I* may be involved in the general

maintenance of islet integrity by promoting cellular proliferation.

Factors that control production of *reg I* product are unknown. Rouquier et al.[6] noted that rat *reg I* is induced early following pancreatic injury and suggested that it was important during the dedifferentiation leading to pancreatic recovery. We recently found that the cellular level of *reg I* gene expression is a specific marker of the state of acinar cell differentiation.[7] Interestingly, Watanabe et al.[3] demonstrated, by means of Northern blot analysis, that although human *reg I* mRNA is not typically expressed in the normal colon or rectum, it is occasionally expressed in colorectal cancers. Ectopic *reg I* mRNA expression in the gastrointestinal tract suggests that it may be a marker of other gastrointestinal epithelial cell differentiation or may even be involved in gastrointestinal tumorigenesis.

The present study was undertaken to localize the production of *reg I* protein in colonic neoplasia, specifically to determine if, as predicted above, it is uniquely expressed in colon cancer cells. We used a monoclonal antibody to *reg I* that has been well characterized[8] and has been used in our laboratory.[21] In our hands, control sections of normal colon did not react with *reg I* antibody, whereas sections of normal pancreas showed *reg I* protein in acinar cells.

Within the sections of colon cancer we observed variable expression of *reg I* protein; 58.9% of all the cancers expressed *reg I*. Sections in which the interface (or transition zone) of normal colonic mucosa with that of neoplasia was observed all stained positive for *reg I*, as did all of the polyps containing carcinoma. The cancerous tissue in all of the polyps similarly stained positive for *reg I*. Molecular analysis of the transition zones from two freshly isolated colon cancers confirmed the presence of both *reg I* mRNA and protein, by Northern and Western blot analysis, respectively. This was not observed in adjacent normal colon. *reg I* positivity did not correlate with histologic grade or 5-year survival, but all Dukes' A tumors were positive for *reg I*.

The pattern of *reg I* positivity thus demonstrated ectopic expression of the protein in association with neoplasia of the colon, suggesting that *reg I* could be a protein with a role in early tumorigenesis. Glands located at a distance from the tumor in these patients were also observed to occasionally express the protein. At its farthest distance away from the tumor, the *reg I* expression was limited to one or two cells at the bottom of the crypt. With increasing proximity to the tumor, more crypts and a greater percentage of these crypt cells expressed the protein, manifested as an upward shift of positivity within the crypt.

reg I protein was present in the eight adenomas harboring cancers. This was significant since adenomas are believed to be an important intermediate stage in the abnormal progression of normal colonic epithelial cells to carcinoma. The detection of *reg I* in all of the adenomas harboring malignancy further supports the implication that *reg I* could be a protein involved in early tumorigenesis.

The earliest step in the development of colorectal neoplasia is believed to be an increase in cellular proliferation within the normally restricted zone at the base of the colonic crypt and the retention of cells capable of proliferation at the top of the colonic crypt.[23] In fact, in vitro labeling of colorectal mucosa has shown an increase in proliferation at the transition zone,[24] as well as in the entire colon of patients at risk.[25] Other investigators have shown that oncogenes such as *c-fos* and *c-jun* are upregulated within the normal-appearing mucosa adjacent to colorectal tumors, and they have suggested that these areas are therefore preneoplastic.[26] Recently, investigators have described the aberrant crypt[27] in normal-appearing mucosa. Aberrant crypts are found in higher frequency in colons at risk for cancer, and they are believed to be the earliest precursor to colorectal neoplasia. To date, there are no consistent histochemical markers for colons at risk for neoplasia.

In our study the strongest positivity for *reg I* was consistently detected at the zone of transition from normal to malignant cells and in all adenomatous glands. This interface is a hot zone for increased cellular proliferation, tumor oncogene overexpression, and aberrant crypt localization. Not all cancers were positive for the protein, nor was it found in normal colonic tissue. This suggests that the production of *reg I* by the colon is a marker of mucosa at risk for neoplasia.

The precise nature of the role that the *reg I* plays in tumorigenesis, however, remains to be elucidated. No hypothesis is available at present regarding the function of *reg I* even in tissues where it is normally expressed. *reg I* was originally isolated in pancreatic juice and was called pancreatic stone protein[11] or pancreatic thread protein.[13] The gene is located at chromosome 2p12, near an area that codes for two important mismatch-repair genes.[28,29] Damage to these genes, *mutS* and *MSH2*, has recently been proposed to be the mechanism of injury leading to hereditary nonpolyposis colon cancer. The proximity of *reg I* to these genes could be a factor in its induction during neoplasia.

The amino acid sequence or *reg I* predicts it to be a calcium-dependent lectin,[2] which is believed to be involved in cell growth and proliferation. Lectins such

as peanut lectin and *Amaranthus caudatus* agglutinin, soybean agglutinin, and *Ricinus communis* agglutinin-1 have been used as exogenous markers for colonic differentiation and neoplasia.[30,31] To our knowledge *reg I* is the only marker described to date that is not typically expressed in the normal colon but is present in areas of neoplastic transformation and could be used as an endogenous marker.

The function of *reg I* protein is still unknown. Original studies on pancreatic stone protein (identical in sequence to *reg I*) showed the N-terminus of the molecule to be important in preventing calcium carbonate precipitation in pancreatic juice.[11,12] In 1988 Terazono et al.[1] described *reg I* as an exocrine product involved in pancreatic islet formation. In 1994 Watanabe et al.[32] showed a direct mitogenic effect of recombinant *reg I* protein in vivo in a model of islet regeneration following pancreatectomy. We have recently shown that the *reg I* gene is induced very early in a model of islet hyperplasia[9] and that the rat and human protein product is mitogenic to pancreatic β-cells and ductular cells in culture.[21] Also, we have shown that in vitro *reg I* gene expression and protein expression inversely correlate with the level of cellular differentiation.[7] Specifically, as AR42J cells differentiate, cellular proliferation decreases, amylase production increases, and *reg I* gene and protein production decrease. It is therefore possible that *reg I* is either a proliferative factor in colorectal mucosa or simply a marker of the state of cellular differentiation. Although in normal areas its genetic expression is inhibited, in areas of dedifferentiation and neoplastic transformation the gene is induced and the protein translated. Both the genetic expression and the protein production of *reg I* are therefore potential useful markers of colonic mucosa at risk for malignant transformation.

CONCLUSION

To confirm the usefulness of *reg I* as a marker for mucosa at risk for neoplasia, further studies are needed. Specifically, colon specimens from patients with genetic dispositions for cancer, such as familial polyposis and Lynch syndrome, should be evaluated. Colons of patients at risk for the development of cancer because of conditions such as ulcerative colitis or other gastrointestinal malignancies involving the pancreas and/or stomach need to be studied. Finally, measurements of local mucosal levels of *reg I* mRNA or protein might have a use in confirming safe margins of resection in colon cancer surgery.

The availability of means to detect *reg I* RNA and protein from small biopsy-sized tissue samples would add to the potential use of *reg I* as a tool in cancer therapy. We have recently described a method of assessing the reverse transcriptase polymerase chain reaction that is applicable to very small biopsy-sized tissue samples[33,34] and could be adapted to *reg I* levels in colon tissue. Also, a fluorometric immunoassay has recently been devised to quantitate pancreatic stone protein providing sensitivity for concentrations ranging from 0.015 to 0.5 μg/ml. The sensitivity for lower concentrations can be enhanced by electrophoretic methods.

REFERENCES

1. Terazono K, Yamamoto H, Takasawa S, Shiga K, Yonekura Y, Tochino Y, Okamoto H. A novel gene activated in regenerating islets. J Biol Chem 1988;263:2111-2114.
2. Unno M, Yonekura H, Nakagawara K, Watanabe T, Miyashita H, Moriizumi S, Okamoto H. Structure, chromosomal localization and expression of mouse *reg* genes *reg I* and *reg II*. J Biol Chem 1993;268:15974-15982.
3. Watanabe T, Yonekura H, Terazono K, Yamamoto H, Okamoto H. Complete nucleotide sequence of human *reg* gene and its expression in normal and tumoral tissue. J Biol Chem 1990;265:7422-7439.
4. De Reggi M, Capon C, Gharib B, Wieruszeski JM, Michel R, Fournet B. The glycan moiety of human pancreatic lithostatine. Eur J Biochem 1995;230:503-510.
5. Perfetti R. Assignment of the human pancreatic regenerating (*reg*) gene to chromosome 2p12. Genomics 1994;20:305-307.
6. Rouquier S, Verdier JM, Iovanna J, Dagorn JC, Giorgi D. Rat pancreatic stone protein in mRNA. J Biol Chem 1991; 266:786-791.
7. Zenilman ME, Magnuson TH, Perfetti R, Swinson K, Shuldiner AR. *Reg* gene expression is inhibited during differentiation of cultured rat acinar cells. Ann Surg (in press).
8. Provansal-Cheylan M, Lusher M, de Caro A, Multigner L, Montalto G, Sarles H, Delaage M. Monoclonal antibodies to pancreatic stone protein: Radioimmunoassay and immunological comparison with trypsin 1. Biochimie 1986;68:1109-1113.
9. Zenilman ME, Perfetti R, Swinson K, Magnuson TH, Shuldiner AR. Pancreatic regeneration (*reg*) gene expression in a rat model of islet hyperplasia. Surgery 1996;119:576-584.
10. Miyaura C, Chen L, Appel M, Alam T, Inman L, Hughes SD, Milburn JL, Unger RH, Newgard CB. Expression of reg/PSP, a pancreatic exocrine gene: Relationship to changes in islet β-cell mass. Mol Endocrinol 1991;5:226-234.
11. de Caro A, Lohse J, Sarles H. Characterization of a protein isolated from pancreatic calculi of men suffering from chronic calcifying pancreatitis. Biochem Biophys Res Commun 1979; 87:1176-1182.
12. Giorgi D, Bernard JP, Rouquier S, Iovanna J, Sarles H, Dagorn JC. Secretory pancreatic stone protein messenger RNA. J Clin Invest 1989;84:100-106.
13. Gross J, Carlson RI, Brauer AW, Margolies MN, Warshaw AL, Wands JR. Isolation, characterization, and distribution of an unusual pancreatic human secretory protein. J Clin Invest 1985;76:2115-2126.
14. Gross J, Brauer AW, Bringhurst RF, Corbett C, Margolies MN. An unusual bovine pancreatic protein exhibiting pH-de-

pendent globule-fibril transformation and unique amino acid sequence. Proc Natl Acad Sci USA 1985;82:5627-5631.
15. Orelle B, Keim V, Dagorn JC, Iovanna JL. Human pancreatitis associated protein: Messenger RNA cloning and expression in pancreatic diseases. J Clin Invest 1992:90:2284-2291.
16. Iovanna J, Orelle B, Keim V, Dagorn JC. Messenger RNA sequence and expression of rat pancreatitis associated protein, a lectin related protein overexpressed during acute experimental pancreatitis. J Biol Chem 1991;266:24664-24669.
17. Iovanna JL, Keim V, Nordback I, Montalto G, Camarena J, Letoublon C, Levy P, Berthezene P, Dagorn JC. Serum levels of pancreatitis associated protein as indicators of the course of acute pancreatitis. Gastroenterology 1994;106:728-734.
18. Lasserre C, Christa L, Simon MT, Vernier P, Brechot C. A novel gene (HIP) activated in human primary liver cancer. Cancer Res 1992;52:5089-5095.
19. Dusetti NJ, Frigerio JM, Fox MG, Swallow DM, Dagorn JC, Iovanna JL. Molecular cloning, genomic organization and chromosomal localization of human pancreatitis associated protein (PAP) gene. Genomics 1994;19:108-114.
20. Vinik AI, Pittenger G, Rafaeloff R, Rosenberg L. Factors controlling pancreatic islet neogenesis. Tumor Biol 1993;14:184-200.
21. Zenilman ME, Magnuson TH, Swinson K, Egan J, Perfetti R, Shuldiner AR. Pancreatic thread protein is mitogenic to pancreatic-derived cells in culture. Gastroenterology 1996; 110:1208-1214.
22. Zenilman ME, Chen J, Magnuson TH, Shuldiner AR. Human PTP/*reg* is mitogenic to primary culture of pancreatic ductal cells. Gastroenterology 1995;108:A402.
23. Boland CR. The biology of colorectal cancer. Cancer 1993; 71(Suppl):4181-4185.
24. Risio M. Cell proliferation in colorectal tumor progression: An immunohistochemical approach to intermediate biomarkers. J Cell Biochem Suppl 1992;16G:79-87.
25. Terpestra OT, van Blankenstein M, Dees J, Eilers GA. Abnormal pattern of cell proliferation in the entire colonic mucosa of patients with colon adenoma or cancer. Gastroenterology 1987;92:704-708.
26. Pandey S, Gordon PH, Wang E. Expression of proliferation-specific genes in the mucosa adjacent to colon carcinoma. Dis Colon Rectum 1995;38:462-467.
27. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG, Jurcisek JA, Stellato TA. Aberrant crypts: Putative neoplastic foci in human colonic mucosa. Cancer Res 1991;51: 1564-1567.
28. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a *mutS* homolog in hereditary nonpolyposis colon cancer. Cell 1993;75:1215-1225.
29. Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell 1993;75:1027-1038.
30. Bolan CR, Martin MA, Goldstein IJ. Lectin reactivities as intermediate biomarkers in premalignant colorectal epithelium. J Cell Biochem Suppl 1992;16G:103-109.
31. Lipkin M. GI cancer: Pathogenesis, risk factors and the development of intermediate biomarkers for chemoprevention studies. J Cell Biochem Suppl 1992;16G:1-13.
32. Watanabe T, Yonemura Y, Yonekura H, Suzuki Y, Miyashita H, Sugiyama K, Moriizumi S, Unno M, Tanaka O, Kondo H, Bone AJ, Takasawa S, Okamoto H. Pancreatic beta-cell replication and amelioration of surgical diabetes by *reg* protein. Proc Natl Acad Sci USA 1994;91:3589-3592.
33. Zenilman ME, Graham W, Tanner K, Shuldiner AR. Competitive reverse transcriptase PCR without an artificial internal standard. Anal Biochem 1995;224:339-346.
34. Zenilman ME, Graham WS. Quantitation of messenger RNA for insulin-like growth factor I receptor in human colonic tissue by quantitative PCR. Cancer Invest (in press).

Discussion

Dr. B. Schirmer (Charlottesville, Va.). Have you ever examined specimens from patients with familial polyposis or rectal segments from patients with ulcerative colitis or villous tumors to see whether there is expression of the *reg* protein in those tissues?

Dr. M.E. Zenilman. Of the polyps we examined, half were villous and half were tubulovillous, and all of them were positive for *reg I*. We are presently going back and looking at fixed specimens from patients with ulcerative colitis and familial polyposis to see whether in areas of dysplasia, which is considered the worrisome precursor, *reg* is expressed there. This would be a very useful marker and tests could be performed relatively quickly in the pathology laboratory.

Dr. J.C. Thompson (Galveston, Tex.). From the way you describe this, I get the impression that you think this gene is some sort of trigger but that it is lost in the neoplastic tissue. Have you tested the actual tumors themselves for message for this gene and, if so, have you noted any correlation between the degree of dedifferentiation and the presence of message in the tumor?

Dr. Zenilman. We are planning a prospective study in which we are going to take sections of specific intervals from the tumor, both circumferentially and longitudinally, and from within the tumor itself. I think *reg I* is a gene product that turns on and then turns off as the neoplasia starts to take over. I am not sure it has anything to do with neoplasia itself other than being a marker. Interestingly, chromosome 2p12, where we localized the gene, is right next to two recently described mismatch-repair genes, mutations that are found in patients with hereditary nonpolyposis colon cancer. *reg I* production may be an effect of those mutations and is therefore a marker of it.

Dr. M. Tobi (Detroit, Mich.). The transition zone is extremely versatile with regard to expression of ectopic-type phenomena. We know that in the fetus we can see the ex-

pression of blood group antigens. Have you examined this area for other ectopic expressions such as, for example, incompatible blood group expressions, and are you planning to study fetal tissues?

Dr. Zenilman. The answer to the first question is no, we have not looked at other ectopically expressed proteins. We have seen, in the mouse, that *reg* gene expression is turned on along with insulin gene expression very early in embryogenesis, before the pancreas is even visible. We also have very preliminary data which suggest that the *reg* gene might be turned on in fetal tissue in the intestine even before birth. As of yet, I am not completely convinced of it, but we have some very fine in situ work that is highly suggestive of it. It might be an embryologic marker for gastrointestinal development.